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Journal OF THE Fisheries Research Board OF Canada

Aspects saisonniers de la fixation de l'épifaune benthique de l'étage infralittoral de l'estuaire du Saint-Laurent[1]

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BOURGET, E., ET G. LACROIX. 1973. Aspects saisonniers de la fixation de l'épifaune benthique de l'étage infralittoral de l'estuaire du Saint-Laurent. J. Fish. Res. Board Can. 30: 867–880.

Ce travail, poursuivi pendant deux saisons (mai–octobre 1969, 1970), avait comme objectif d'étudier les aspects dynamiques de la colonisation des substrats durs par l'épifaune benthique. Les stations d'étude sont échelonnées sur une distance de 200 km, le long d'un gradient de salinité s'étendant de 15 à 27‰. Bien que plus de 60 espèces furent identifiées, trois espèces seulement sont abondantes: *Polydora ciliata*, *Balanus balanoides* et *Balanus crenatus*. Les périodes de colonisation sont de courte durée et les processus de colonisation sont restreints aux mois de juin, juillet et août. Les populations atteignent leur densité maximale pendant la fixation et décroissent graduellement ensuite. Cette réduction est attribuable à la compétition intraspécifique et interspécifique pour l'espace, à l'accumulation de sédiments, et aux facteurs hydrographiques, à l'automne. Le développement de la communauté reflète les contrastes physiques saisonniers très accentués de ce milieu subarctique plutôt qu'une vraie succession biotique. La communauté épibenthique de l'estuaire du Saint-Laurent est biologiquement harmonisée pendant l'été, mais davantage soumise à des contrôles physiques durant l'automne et l'hiver.

BOURGET, E., ET G. LACROIX. 1973. Aspects saisonniers de la fixation de l'épifaune benthique de l'étage infralittoral de l'estuaire du Saint-Laurent. J. Fish. Res. Board Can. 30: 867–880.

This work conducted during two seasons (May–October 1969, 1970) was designed to study the dynamic aspects of the settlement of benthic epifauna on hard substrata. The stations were distributed on a 200-km distance, the salinity gradient ranging from 15 to 27‰. Although over 60 species were identified on the plates, the analysis shows that only three of these are

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abundant: *Polydora ciliata*, *Balanus balanoides*, and *Balanus crenatus*. In general, the settlement period of the species is very short and most of the settling processes are concentrated in June, July, and August. The populations reached a peak of abundance during settlement and decreased regularly afterwards. This decrease is due mainly to intraspecific and interspecific competition for space, accumulation of sediment, and hydrodynamic factors in autumn. The marked physical seasonality of this subarctic environment, more than true biotic succession, is reflected in the development of the community: during the summer, the community is biologically accommodated, whereas in autumn and winter it is physically controlled.

Reçu le 11 décembre, 1972

CONTRAIREMENT au zoobenthos du golfe Saint-Laurent, objet de plusieurs études faunistiques et écologiques, celui de l'estuaire du Saint-Laurent ne fut l'objet que d'explorations faunistiques partielles. C'est donc dans le but de fournir une information à la fois faunistique et écologique concernant les organismes épibenthiques de l'estuaire du Saint-Laurent que cette étude fut entreprise.

Les objectifs suivants sont poursuivis dans ce travail: a) connaître la distribution des organismes épibenthiques le long de l'Estuaire; b) déterminer les périodes de fixation et d'abondance des organismes; c) évaluer l'abondance saisonnière des organismes dominants; d) déceler les facteurs qui provoquent des changements dans la communauté épibenthique.

Les objets immergés durant une certaine période dans le milieu marin sont généralement sujets à une forte colonisation par les organismes sessiles et sédentaires. Sur le plan méthodologique, nous avons utilisé cette propriété pour recueillir les organismes et analyser leurs interactions, en leur fournissant des surfaces vierges qu'ils pouvaient envahir à un rythme proportionnel à leur abondance.

TABLEAU 1. Calendrier des prélèvements et des remplacements des plaques immergées en 1969. Les numéros désignent des séries de plaques immergées simultanément, chaque numéro correspondant à une plaque.

Date	Immersion	Prélèvement
11 mai	1a 1b 1c 1d 1e 1f 1g 1h 1i 1j 1k 1*l*	
26 mai		1a
9 juin	3a 3b 3c 3d 3e	1b
23 juin		1c
7 juillet	5a 5b 5c 5d	1d 3a
21 juillet		1e
4 août	7a 7b 7c	1f 3b 5a
18 août		1g
30 août	9a 9b	1h 3c 5b 7a
13 sept.		1i
27 sept.	11a	1j 3d 5c 7b 9a
11 oct.		1k
25 oct.		1*l* 3e 5d 7c 9b 11a

Matériel et méthodes

Les échantillonneurs utilisés pour recueillir la faune sessile et sédentaire ont déjà été décrits (Bourget et Lacroix 1971). A chaque station, des plaques d'amiante de 0.5 × 10 × 15 cm furent immergées pendant des périodes d'une durée de 0.5 à 6 mois. Les prélèvements et les remplacements des plaques eurent lieu régulièrement à tous les quatorze jours, suivant le calendrier donné au tableau 1. Ce protocole nous a permis de suivre l'évolution des associations épibenthiques établies sur des plaques immergées en mai, juin, juillet jusqu'au dernier retrait à la fin d'octobre.

La localisation des stations apparaît à la figure 1. Quatre emplacements furent retenus: Pointe-aux-Orignaux, près de Rivière-Ouelle (station 1), Gros-Cacouna (station 2), Cap-Marteau, près de Trois-Pistoles (station 3) et Pointe-Métis (station 4). A Cap-Marteau (station 3), des échantillonneurs verticaux (station 3V) et horizontaux (station 3H) furent immergés.

Hydrographie

Les caractéristiques physico-chimiques des milieux estuariens et particulièrement de l'estuaire du Saint-Laurent sont très variables. Les caractéristiques physiques générales de l'Estuaire ont été décrites (Gaudry 1938; Nadeau 1938) et permettent d'expliquer partiellement les extrêmes et la complexité de certaines propriétés physiques de l'Estuaire. Il nous semble donc important d'analyser quelques-uns des principaux facteurs physiques aux stations d'étude, soit la salinité, la température de l'eau et la turbidité.

SALINITÉ

Les mesures conductimétriques de salinité furent faites dans des échantillons d'eau recueillis au moins deux fois par mois, à marée basse et haute, à toutes les stations d'étude. Elles furent déterminées dans des échantillons d'eau prélevés plusieurs fois par semaine à Pointe-Métis et sporadiquement à Cap-Marteau.

La figure 2 illustre les variations saisonnières de la salinité aux stations d'étude en 1969 et 1970. Toutes les salinités minimales furent observées en mai lorsque l'apport d'eau douce par les rivières en crue est considérable. Par ailleurs, en 1969, à toutes les stations, à l'exception de Pointe-aux-Orignaux (station 1), les salinités maximales sont celles de septembre, avant que les pluies automnales n'augmentent le débit des rivières et ne réduisent la salinité des eaux littorales. A Pointe-

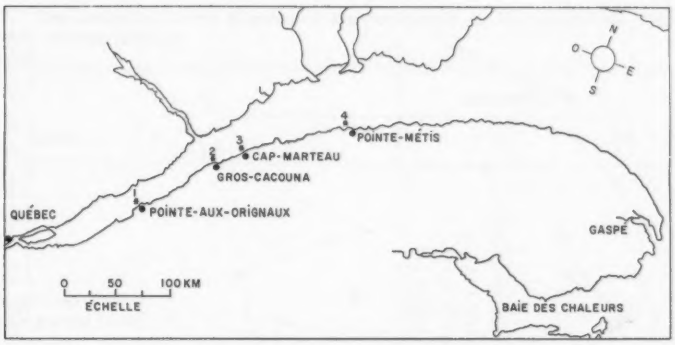

FIG. 1. Situation géographique des quatre stations d'étude.

aux-Orignaux (station 1), les salinités les plus élevées se retrouvent au mois d'août. En 1970, ces salinités maximales surviennent en juillet à Cap-Marteau (station 3) et en août à Pointe-Métis (station 4).

L'amplitude totale de la salinité est faible durant l'hiver. Les extrêmes enregistrés du mois de novembre au mois d'avril à Pointe-Métis (station 4) furent de 23.18‰ et 30.36‰, la moyenne étant de 26.39‰.

TEMPÉRATURE

Les observations de température furent faites au moment du prélèvement des échantillons d'eau. Les variations saisonnières de la température de 1969 et 1970 sont illustrées à la figure 2. Les températures printanières sont basses en 1969, les eaux se réchauffent progressivement jusqu'à la fin de juillet aux stations de Pointe-aux-Orignaux (station 1), Gros-Cacouna (station 2), Pointe-Métis (station 4) — à Cap-Marteau (station 3), le maximum fut atteint à la mi-juin — puis elles se refroidissent graduellement par la suite. En 1970, à la station de Cap-Marteau (station 3) on note que le taux du réchauffement printanier est beaucoup plus lent qu'en 1969. Par ailleurs, c'est par son réchauffement printanier tardif que la station de Pointe-Métis (station 4) se distingue davantage en 1970. La température varie peu durant l'hiver: du mois de décembre à la fin du mois de mars, la température est demeurée constante à –1.1 C.

TURBIDITÉ

Nota et Loring (1964), en se basant sur des mesures effectuées à l'aide d'un disque de Secchi, ont subdivisé l'estuaire en zones de plus ou moins grande turbidité. Selon ces divisions, les stations de Pointe-aux-Orignaux (station 1) et Gros-Cacouna (station 2) sont localisées dans une zone de transparence inférieure à 2 m, la station de Cap-Marteau (station 3), dans une zone de 2 à 5 m de transparence et enfin la station de Pointe-Métis (station 4), aux limites de la zone de 2 à 5 m et de celle de 5 à 10 m de transparence. Nos observations aux stations d'étude, au cours des mois de juillet et août 1970, coïncident avec celles de ces deux auteurs (Bourget et Lacroix 1972).

Sur le plan saisonnier, la transparence était plus faible au printemps et à l'automne. Pardé (1948), mentionne que la décharge maximale se produit entre la mi-mai et la mi-juin et qu'un second maximum survient à la fin d'octobre. Durant ces périodes, l'apport direct des rivières en crue et l'érosion de la zone intertidale par les glaces (Dionne 1968) à la fin de l'hiver contribue à augmenter les matières en suspension dans l'eau et à réduire davantage la transparence.

Distribution et abondance relative de la faune épibenthique

L'analyse de la communauté épibenthique de chacune des stations portera essentiellement sur quelques espèces sédentaires ou sessiles abondantes (*Polydora ciliata*, *Balanus balanoides*, *Balanus crenatus*). La technique d'échantillonnage utilisée a pu conduire à une sous-évaluation de la densité des organismes vagiles (Nématodes, Copépodes harpacticoides, Amphipodes), puisqu'ils peuvent fuir au moment du prélèvement des plaques. Ils ne seront donc présentés que sommairement.

Tous les organismes recueillis sur les plaques ont été dénombrés. Leur abondance relative ainsi que leur distribution apparaissent au tableau 2.

FAUNE VAGILE

Nous avons trouvé sur les plaques au moins 22 espèces de Nématodes (tableau 2). La plupart ne sont représentées que par quelques individus. Une seule espèce est importante: *Theristus acer*. Ainsi, en 1969, à Cap-Marteau (station 3V), sur une plaque immergée le 1er mai et prélevée au mois d'août, nous observons une densité de Nématodes de 261 ind./dm² constituée par *Theristus acer* dans une proportion de 91%.

Les Copépodes harpacticoïdes sont également abondants sur les plaques. Les valeurs élevées (12,343 ind./dm², 2589 ind./dm², obtenues respectivement aux stations 3V et 3H de Cap-Marteau) sont atteintes sur des plaques immergées en mai et prélevées en août lorsque le développement épibenthique est important.

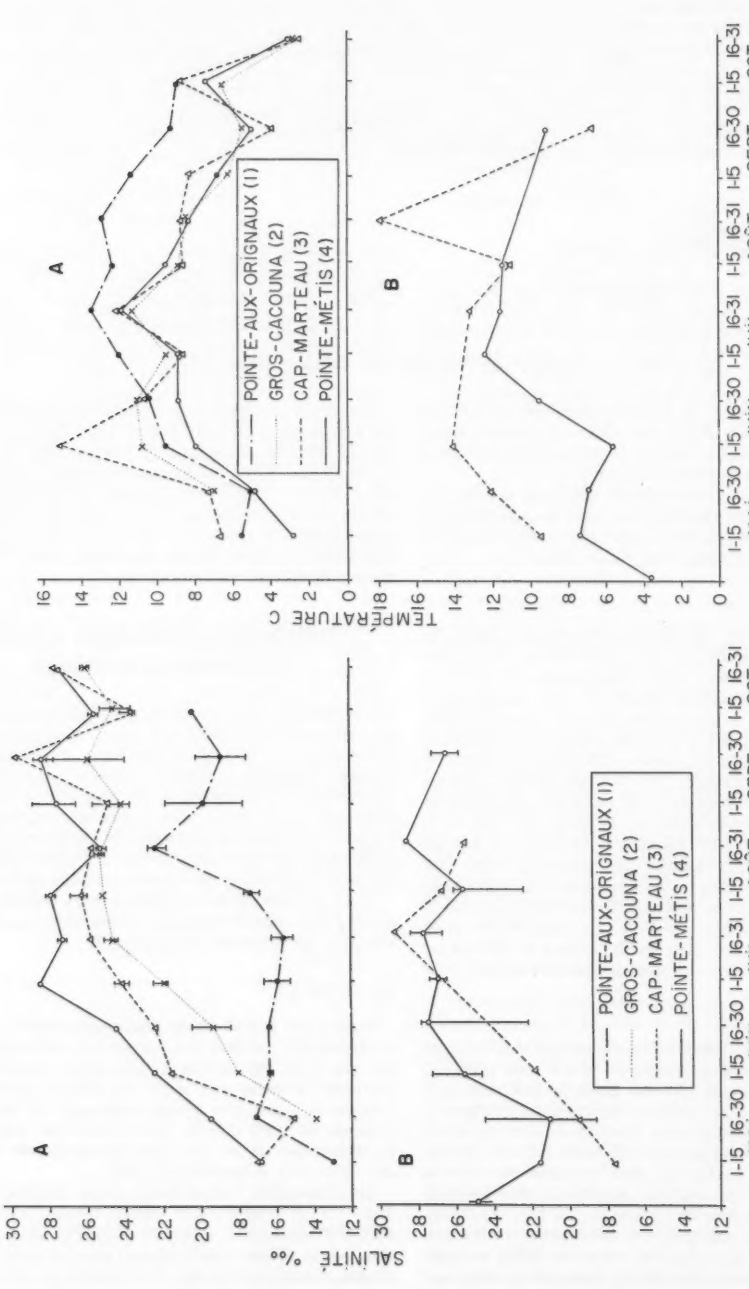

FIG. 2. Variations saisonnières de la salinité et de la température moyenne, en 1969 (*en haut*), en 1970 (*en bas*). Les traits verticaux représentent l'amplitude; (●) Pointe-aux-Orignaux; (×) Gros-Cacouna; (△) Cap-Marteau; (○) Pointe-Métis.

TABLEAU 2. Abondance relative et distribution des organismes sur les plaques des échantillonneurs immergés aux stations d'étude.

Taxon	Stations[a] 1	2	3V	3H	4
PROTOZOA					
Foraminifera	+	+	+	+	+
CNIDARIA					
Hydroida					
Obelia longissima (Pallas)	–	–	++	++	–
Sertularella pumila Linné	–	–	–	+	–
*ASCHELMINTHES					
Nematoda					
Araeolaimus sp.	–	–	–	–	+
Bathylaimus sp.	–	–	+	–	–
Bradylaimus sp.	+	–	–	+	–
Chromadora axi Gerlach	–	+	+	–	–
Chromadora nudicapitata Bastian	–	+	+	–	–
Chromadora sp.	–	+	+	–	–
Chromadorita sp.	–	+	+	–	–
Cyatholaimus sp.	–	–	–	–	+
Dychromadora sp.	–	+	+	–	–
Enoplolaimus sp.	–	–	+	–	–
Enoplus communis Bastian	–	–	–	+	–
Enoplus sp.	–	–	+	–	–
Mesotheristus	–	–	+	–	–
Monhystera disjuncta Bastian	–	+	–	–	–
Monhystera sp.	–	+	–	–	–
Monoposthia costata Bastian	–	++	+	+	–
Neochromadora sp.	–	–	–	+	–
Neonyx sp.	–	–	–	+	–
Nudora sp.	–	–	+	–	–
Oncholaimidae non identifiés	–	+	–	+	–
Prochromadorella sp.	–	+	–		–
Rhabditis marina Bastian	–	–	–	–	+
Steineridora loricata Steiner	–	+	–	–	–
Theristus acer Bastian	–	+++	+++	+	+
Theristus sp.	–	+	+	–	–
Tripyloides gracilis Ditlevsen	–	–	+	–	–
Tripyloides sp.	–	+	+	–	–
Nématodes non identifiés	+	–	–	–	–
BRYOZOA					
Celleporaria sp.	–	–	–	+	–
Tegella armifera (Hincks)	–	–	–	+	–
*MOLLUSCA					
Gasteropoda					
Lacuna pallidula neritoidea Gould	–	+	+	–	–
Lacuna vincta (Montagu)	–	+++	+	–	–
Littorina obtusata Linné	–	+	+	–	–
Littorina saxatilis (Olivi)	–	+	–	–	–
Margarites helicinus (Phipps)	–	+	–	–	–
Pelecypoda					
Mytilus edulis Linné	–	–	+	+	++

(*à suivre*)

TABLEAU 2. Abondance relative et distribution des organismes sur les plaques des échantillonneurs immergés aux stations d'étude — *(fin)*.

Taxon	Stations[a] 1	2	3V	3H	4
*ANNELIDA					
Polychaeta					
Dysponetus pygmaeus Levinsen	–	+	–	–	–
Harmothoe imbricata (Linné)	–	–	–	+	–
Nereis sp.	+	+	–	–	+
Phyllodoce maculata (Linné)	–	+	+	+	+
Polydora ciliata Claparède	+	+	+++	+++	+
Spionidae non identifiés	–	–	–	+	–
CRUSTACEA					
*Ostracoda					
Candona caudata Kaufmann	–	–	+	–	–
Cypricercus fuscatus (Sars)	–	–	–	–	+
Cytheridea papillosa Bosquet	–	+	–	–	–
Cytherois sp.	–	+	–	–	–
Cytherura nigrescens (Baird)	+	++	++	+	–
Cytherura similis Sars	–	+	–	–	–
Hemicythere emarginata Sars	–	–	–	–	+
Hemicythere sp.	+	+	–	–	–
Paracytherois arcuata (Brady)	–	+	–	–	–
Sclerochilus contortus (Norman)	–	+	+	+	–
*Copepoda	+	+			
Harpacticoida	+	+	+++	+++	+++
*Cirripedia					
Balanus balanoides (Linné)	+	–	+	+	++
Balanus crenatus Bruguière	+++	–	+	++	++
*Amphipoda					
Calliopius laeviusculus (Krøyer)	–	+	+	–	++
Gammarus oceanicus Sergestrale	–	+++	+++	++	++
Gammarus lawrencianus Bousfield	+	+	+	–	+
Ischyrocerus anguipes Krøyer	–	+	++	++	+++
Pontogeneia inermis (Krøyer)	–	–	+	+	++
Caprella	–	+	+	++	–
ARACHNIDA					
Acarina	++	+	–	+	+

[a]+++: très abondante; ++: abondante; +: présente; –: absente.
*Groupes utilisés pour le calcul des "indices de diversité."

De plus, nous avons trouvé sur nos plaques plusieurs espèces d'Amphipodes gammarides. Deux de ces espèces seulement sont abondantes: *Gammarus oceanicus* et *Ischyrocerus anguipes*. Les densités maximales de 2, 22, 49, 23 et 33 ind./dm² ont été observées respectivement à Pointe-aux-Orignaux (station 1), Gros-Cacouna (station 2), Cap-Marteau (stations 3V et 3H) et Pointe-Métis (station 4).

En somme, la plupart des espèces vagiles identifiées ne sont représentées que par quelques individus. Seulement quelques espèces sont abondantes. Ces espèces, de même que les Harpacticoïdes, sont abondantes lorsque le développement des algues et/ou d'une population sessile (*P. ciliata*) vient accroître la complexité topographique de l'habitat.

FAUNE FIXÉE

Hydrozoaires — Sertularella pumila et *Obelia longissima* sont les deux espèces d'hydrozoaires récoltées sur les plaques. *Obelia longissima* se rencontre sur les échantillonneurs verticaux et horizontaux à Cap-Marteau (station 3) à compter du 18 août, alors que *S. pumila* ne fut observée que sur les échantillonneurs horizontaux, le 13 septembre.

En 1970, l'espèce *O. longissima* fut observée à une seule occasion sur les plaques verticales immergées de mai à septembre à Cap-Marteau (station 3) et la présence de *S. pumila* ne fut pas relevée sur les plaques.

Gastéropodes — Les Gastéropodes étaient représentés par cinq espèces: *Lacuna pallidula neritoides, Lacuna vincta, Littorina saxatilis, Littorina obtusata, Margarites helicinus.*

Examinons d'abord les résultats de 1969. L'espèce *Lacuna vincta* est relativement abondante à Gros-Cacouna (station 2), où elle constitue plus de 90% des Gastéropodes recueillis. A Cap-Marteau (station 3), par ailleurs, elle ne forme plus que 40% des Gastéropodes, les autres individus appartenant à l'espèce *Lacuna pallidula.*

Les densités maximales de 26 ind./dm², 4.66 ind./dm², 8 ind./dm² et 1.33 ind./dm² obtenues respectivement à Gros-Cacouna (station 2), à Cap-Marteau (stations 3V et 3H) et à Pointe-Métis (station 4) montrent bien la faible abondance des Gastéropodes sur les plaques. Les densités les plus élevées sont toutes notées sur des plaques immergées durant une période variant de 2 à 4 semaines. Cette observation indique que la colonisation est meilleure sur des surfaces dont le développement algal est amorcé mais pas trop important. En 1970, la fixation sur les plaques immergées en mai est très faible: 2 *L. vincta*/dm² à Cap-Marteau (station 3V) sur une plaque prélevée le 1er septembre.

Pélécypodes — Sur nos plaques, une seule espèce appartenant à ce taxon fut identifiée. Il s'agit de *Mytilus edulis* dont les juvéniles étaient présents à Cap-Marteau (station 3V et 3H) et à Pointe-Métis (station 4), seuls endroits où les adultes sont abondants dans le littoral.

Cette espèce est présente à compter du 4 août à Cap-Marteau (stations 3V et 3H), et à la fin d'août à Pointe-Métis (station 4). La durée de la fixation de *Mytilus edulis* n'est que de 15 jours à cette station. A Pointe-Métis (station 4), la densité atteint 43 ind./dm² sur une plaque immergée en juin et retirée le 30 août, alors qu'à Cap-Marteau (station 3), les densités notées sur les échantillonneurs horizontaux et verticaux sont inférieures à 3 ind./dm².

En 1970, la fixation de *Mytilus edulis* est limitée à un seul individu, trouvé sur une plaque prélevée en septembre, à Pointe-Métis (station 4).

Ostracodes — Dix espèces d'Ostracodes sont présentes sur les plaques (tableau 2). *Cytherura nigrescens* est la plus abondante, les autres n'étant récoltées qu'en très faibles quantités et très sporadiquement.

Les Ostracodes apparaissent au début de juillet aux stations de Cap-Marteau (stations 3V et 3H), le 24 juillet à Gros-Cacouna (station 2), et au début d'août à Pointe-aux-Orignaux (station 1). Les densités sont très faibles: la densité maximale n'atteint que 22 ind./dm² sur les plaques immergées en mai et prélevées à la fin d'août à Gros-Cacouna (station 2). On observe une augmentation progressive de leur abondance sur les plaques jusqu'à la fin d'août, puis une diminution graduelle de cette densité durant les mois de septembre et octobre.

En 1970, la présence des Ostracodes est très sporadique. Sur les plaques immergées en mai à Pointe-Métis (station 4), nous avons trouvé une densité de 1 ind./dm² sur une plaque prélevée à la fin de juillet et une densité de 4 ind./dm² sur une plaque retirée au début d'octobre.

Annélides sédentaires — *Polydora ciliata* est présente à toutes les stations, mais elle est particulièrement abondante à Cap-Marteau (station 3), où elle constitue une grande partie de la biomasse animale.

La période de fixation intensive des larves est de courte durée: elle s'étend de la mi-juin à la mi-juillet. Néanmoins, on trouve quelques larves à compter du 1er juin à Gros-Cacouna (station 2) et à Cap-Marteau (station 3H); après la période de fixation intensive, à Pointe-aux-Orignaux (station 1), à Gros-Cacouna (station 2) et à Cap-Marteau (station 3V) (Fig. 3). C'est à Cap-Marteau (station 3) que sont notées les densités les plus élevées. La densité maximale de 2661 ind./dm² est observée sous une plaque horizontale immergée en mai et prélevée le 7 juillet. Par ailleurs, la fixation maximale observée sur les plaques verticales n'est que de 393 ind./dm². Cette fixation accrue sous les plaques horizontales fut observée par plusieurs chercheurs (Visscher 1928; Visscher et Luce 1928; Hopkins 1935; Pomerat et Reiner 1942; Symon-Papyn 1965 et Sentz-Braconnot 1966), unanimes à expliquer ce phénomène surtout par la faible exposition à la lumière.

A Pointe-aux-Orignaux (station 1), à Gros-Cacouna (station 2) et à Pointe-Métis (station 4), les densités maximales sont respectivement de 2.66, 1.33 et 28.66 ind./dm².

Aussitôt terminée la période de fixation des larves de *Polydora*, on note une diminution graduelle des individus fixés: à Cap-Marteau (station 3V), la densité passe de 392 ind./dm² le 21 juillet, à 191 ind./dm² au début de septembre; à Cap-Marteau (station 3H), la densité, d'une valeur de 2661 ind./dm² le 7 juillet, est réduite à 633 ind./dm² à la mi-octobre (Fig. 4). Cette diminution d'abondance s'observe aussi sur les plaques immergées au début de juin.

En 1970, l'établissement de la population de *P. ciliata* diffère de celui de l'année précédente; la fixation est plus tardive d'environ quinze jours; nous constatons égale-

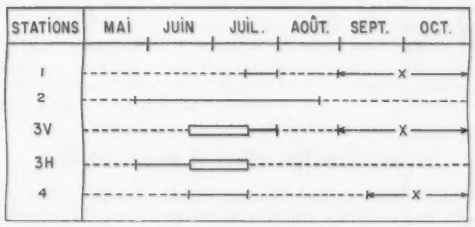

FIG. 3. Période de colonisation des plaques par les larves de *Polydora ciliata*, en 1969. Le trait discontinu indique l'absence d'individus; le trait continu, une faible abondance; le rectangle, une période de forte abondance; le X, l'absence de résultats.

ment une diminution sensible de la densité des individus fixés (tableau 3).

A Cap-Marteau (station 3H), une plaque immergée au début de mai et prélevée au début de juillet supportait 2661 ind./dm² comparativement à 1282 ind./dm² sur une plaque immergée en juin et prélevée à la même date. Le même phénomène se produit à la station 3V: les plaques furent exposées également à l'assaut des larves — la fixation a débuté à la mi-juin — et la plaque immergée depuis plus longtemps portait le plus d'individus. Ces résultats suggèrent qu'un facteur agit pour favoriser la fixation de *P. ciliata* sur des plaques immergées durant une longue période.

Cirripèdes: *Balanus balanoides* — L'examen des densités de *B. balanoides* indique clairement que celles-ci sont faibles à Pointe-aux-Orignaux (station 1), à Cap-Marteau (stations 3V et 3H) et qu'elles sont plus fortes à Pointe-Métis (station 4). Par ailleurs, cette espèce est absente de la station Gros-Cacouna (station 2).

A Pointe-Métis (station 4), seul endroit où l'espèce est abondante, la fixation intensive ne dure que deux semaines, soit de la mi-juillet au début d'août (Fig. 5). Durant cette période, la densité maximale a atteint 113.33 ind./dm² sur une plaque immergée le 7 juillet et prélevée le 4 août. A Pointe-aux-Orignaux (station 1) et à Cap-Marteau (stations 3V et 3H) les densités maximales atteintes furent respectivement de 4.66, 0.66 et 3.33 ind./dm². La période de fixation de *B. balanoides* fut relativement tardive: elle a débuté à la mi-juillet à toutes les stations, à l'exception de la station de Cap-Marteau (station 3V) où un individu s'est fixé du 7 au 21 juillet.

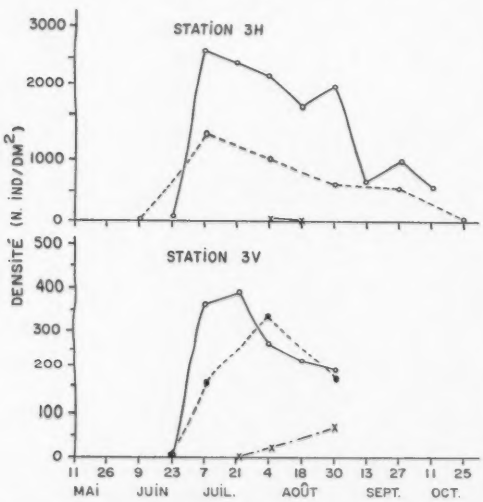

FIG. 4. Variations saisonnières de *Polydora ciliata* à Cap-Marteau (stations 3V et 3H), en 1969, d'après les plaques immergées en mai (●), juin (○) et juillet (×), et prélevées à des intervalles réguliers de 14 jours.

En 1970, aucun individu de *B. balanoides* ne fut récolté à Cap-Marteau (station 3) et à Pointe-Métis (station 4).

L'absence quasi totale de *B. balanoides* sur les plaques de Pointe-aux-Orignaux (station 1) et son absence totale sur le littoral au même endroit sont probablement liées aux conditions hydrographiques caractérisant ce secteur de l'estuaire. En effet, en 1969, la salinité moyenne pour la période mai-août ne fut que 15.7‰. Il est peu probable que les individus de Pointe-aux-Orignaux (station 1) puissent survivre aux salinités printanières, bien inférieures à la salinité moyenne. De plus, comme nous le verrons, les individus qui s'y installent sont rapidement enfouis sous une épaisse couche de sédiments.

Par ailleurs, la faible abondance de *B. balanoides* sur les plaques aux autres stations d'étude trouve son explication dans la distribution bathymétrique de cette espèce: *B. balanoides* est typique de la zone intertidale, et très abondante depuis la ligne des hautes marées moyennes jusqu'aux basses marées (Moore 1935). Cette distribution est maintenue grâce au comportement des larves de *B. balanoides* qui, comme celles de plusieurs organismes intertidaux, sont photopositives, au cours de leur dernier stade larvaire (Runnström 1925). Néan-

TABLEAU 3. Densité de *Polydora ciliata* (N. ind./dm²) sur des plaques immergées au début de mai et prélevées après quatre périodes d'immersion de durées différentes, à Cap-Marteau (station 3V) en 1969 et 1970.

Année	1er mois	2ième mois	3ième mois	4ième mois
1969	0	365	263	191
1970	0	7	101	70

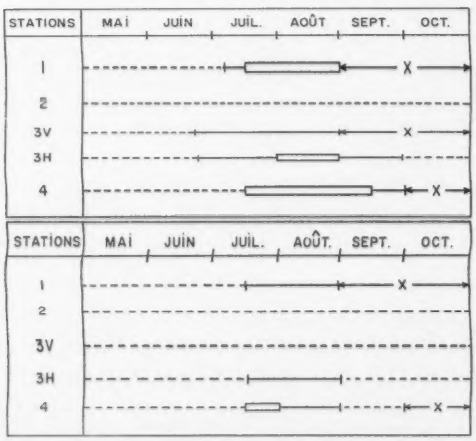

FIG. 5. Période de colonisation des plaques par *Balanus balanoides* (*en bas*) et *B. crenatus* (*en haut*) en 1969. Le trait discontinu, indique l'absence d'individus; le trait continu, une faible abondance; le rectangle, une période de forte abondance; le ×, l'absence de résultats.

moins, l'espèce se retrouve assez couramment dans l'infralittoral jusqu'à environ 9 m de profondeur (Bousfield 1954).

A Pointe-Métis (station 4), sur les plaques immergées en mai, la densité maximale de 60.66 ind./dm² est atteinte deux semaines après le début de la fixation. Par la suite, on constate une diminution graduelle de la densité des individus.

Cirripèdes. Balanus crenatus — Sur le plan géographique, *Balanus crenatus* est présente à Pointe-aux-Orignaux (station 1), à Cap-Marteau (stations 3V et 3H) et à Pointe-Métis (station 4). Soulignons son absence de Gros-Cacouna (station 2) — c'était aussi le cas de *B. balanoides* — où certaines conditions locales non identifiées semblent empêcher sa fixation.

Balanus crenatus est très abondante à Pointe-aux-Orignaux (station 1) et à Pointe-Métis (station 4), où les densités maximales obtenues sont respectivement de 699 ind./dm² sur une plaque immergée de juin à septembre et de 454 ind./dm² sur une plaque immergée durant le mois de juillet et prélevée au début d'août. A Cap-Marteau (station 3V et 3H), les densités maximales sont respectivement de 3.33 et 54.66 ind./dm².

Sur le plan saisonnier, la période de fixation de *B. crenatus* à Pointe-aux-Orignaux (station 1) et à Pointe-Métis (station 4) débute à la mi-juillet (Fig. 5), alors qu'à Cap-Marteau (station 3), la fixation débute plus tôt, vers la mi-juin. A Pointe-aux-Orignaux (station 1), il est impossible de déterminer exactement la date d'arrêt de la fixation parce que les plaques furent perdues au mois de septembre. Par ailleurs, à Cap-Marteau (station 3) et à Pointe-Métis (station 4), l'arrêt de la fixation se produit à la fin de septembre.

En 1969, la période de fixation de *B. crenatus* débute au moment où la température de l'eau atteint 11 C. A Cap-Marteau (station 3H), où la fixation débute tôt en saison, on remarque deux pics de température supérieurs à 11 C. L'un coïncide avec le début de la fixation des larves cypris et le second avec le début de la fixation intense. En 1970, le début de la période de fixation des quelques individus observés coïncide grossièrement avec ce seuil de 11 C. La salinité aux stations d'étude n'influence ni la période, ni l'intensité de la fixation de *B. crenatus*, puisque la densité des individus est élevée aux stations de forte et faible salinité.

Aussitôt la fixation maximale atteinte sur les plaques immergées en mai, juin et juillet, on note une diminution graduelle de la densité de *B. crenatus* sur les plaques à mesure que la saison progresse (Fig. 6).

A Pointe-aux-Orignaux (station 1), la population de Cirripèdes ne fut pas suivie assez longtemps pour qu'on puisse noter une décroissance de la densité comme à Pointe-Métis (station 4). Toutefois, la rareté des Cirripèdes dans le milieu nous amène à croire qu'une telle décroissance doit avoir lieu.

Nous avons estimé l'accumulation de sédiments sur des Cirripèdes fixés à des plaques immergées au début de mai aux stations de Pointe-aux-Orignaux (station 1) et Pointe-Métis (station 4). Il ressort de nos observations qu'à la station de Pointe-aux-Orignaux (station 1), l'accumulation de sédiments est très importante. L'enfouissement des Cirripèdes est beaucoup plus grand à Pointe-aux-Orignaux (station 1) qu'à Pointe-Métis (station 4), par suite de l'extrême abondance des matières en suspension dans les parages de Pointe-aux-Orignaux (station 1). Cet enfouissement est d'ailleurs d'autant plus important que le temps d'immersion est grand (tableau 4). Cette population fut malheureusement suivie sur une trop courte période pour qu'on puisse noter l'effet léthal de cette accumulation graduelle de sédiment sur la population. A l'automne toutefois, le brassage accru des eaux augmente considérablement la quantité de matières en suspension et il est probable que l'accumulation de sédiment est beaucoup plus forte. La sédimentation nous semble donc très importante et pourrait à longue échéance causer une forte mortalité de la population à Pointe-aux-Orignaux (station 1).

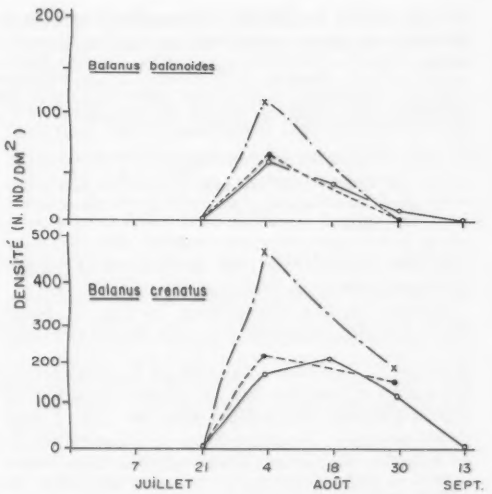

FIG. 6. Variations saisonnières de *Balanus balanoides* et *Balanus crenatus* à Pointe-Métis (station 4), en 1969, d'après les plaques immergées en mai (●), juin (○) et juillet (×), et prélevées à des intervalles réguliers de 14 jours.

Les densités obtenues en 1970 à Pointe-Métis (station 4) sont plus faibles que celles obtenues en 1969. Par ailleurs, la station de Cap-Marteau (station 3V) est relativement pauvre en *B. crenatus* et leur densité diffère peu d'une année à l'autre (tableau 5).

Diversité de la faune épibenthique

L'indice de diversité de la communauté épibenthique pendant chaque période d'immersion a été calculé au moyen de la formule mise au point par Fisher et al. (1943):

$$\alpha = \frac{N\ (1-x)}{x}$$

où α est l'indice de diversité, N le nombre d'organismes fixés sur une plaque, x exprime une relation logarithmique entre N et S (S étant le nombre d'espèces fixées sur la plaque) obtenue de la figure 125 de Williams (1964). L'écart-type (σ) de chaque valeur de l'indice de diversité a été calculé au moyen de l'expression

$$\sigma = \sqrt{\frac{\alpha^3 \left\{(N+\alpha)^2 \log_e \frac{2N+\alpha}{N+\alpha} - \alpha N\right\}}{(SN + S - N)^2}}$$

de Fisher et al. (1943), pour le calcul de l'écart-type d'une seule valeur de l'indice de diversité.

Certains groupes d'organismes (tableau 2) furent exclus du calcul des indices, soit parce que les spécimens ne furent pas identifiés (Protozoaires, Copépodes *harpacticoïdes et Acariens), soit parce qu'il est impossible* de déterminer le nombre précis d'individus (Cnidaires et Bryozoaires).

Il ressort du calcul des indices (tableau 6) que sur les plaques immergées en mai, la station de Gros-Cacouna (station 2) présente la plus grande diversité. Cette grande diversité est la conséquence du nombre élevé des espèces de Nématodes.

A Gros-Cacouna (station 2), les indices de diversité décroissent avec la durée de l'immersion, contrairement à Pointe-Métis (station 4) et Cap-Marteau (stations 3V et 3H) où ils n'ont cessé de croître jusqu'à la fin de la saison. Ces faits suggèrent que des modes de fixation différents régissent ces deux communautés.

En effet, la fixation à Gros-Cacouna (station 2) est caractérisée par un nombre peu élevé d'espèces. La densité est maximale au mois d'août et régresse jusqu'à la fin de la saison. Le patron diffère à Pointe-Métis (station 4) où le nombre de spécimens atteint encore un maximum en août, mais où le nombre d'espèces augmente avec la durée de l'immersion.

A Cap-Marteau (station 3), tant sur les plaques verticales que sur les plaques horizontales, la situation ressemble à celle de Pointe-Métis (station 4): on y remarque une diminution constante du nombre d'individus, conséquence de la diminution du nombre de *P. ciliata*, et une augmentation du nombre d'espèces, surtout attribuables aux Nématodes.

TABLEAU 4. Variation temporelle de la sédimentation sur des Cirripèdes fixés sur des plaques immergées en mai et prélevées à différentes dates, en août. Degrés de sédimentation: (1) accumulation de sédiments autour de la base et des parois; (2) accumulation de sédiments autour de la base, des parois et sur les opercules; (3) enfouissement total sous les sédiments.

			4 août	18 août	30 août
	Nombre d'individus		*34*	*263*	*339*
Station 1	% d'individus par degré de sédimentation	1	9	12.1	44.8
		2	0	5	17
		3	0	3	3.2
	Nombre d'individus		338	377	189
Station 4	% d'individus par degré de sédimentation	1	3.2	6.8	12.2
		2	0.3	0.6	1.3
		3	0	0	0

TABLEAU 5. Densité (N. ind./dm²) de *Balanus crenatus* sur des plaques immergées au début de mai et prélevées après cinq durées d'immersion, à Cap-Marteau (station 3V) et Pointe-Métis (station 4), en 1969 et 1970.

	Année	1er mois	2e mois	3e mois	4e mois	5e mois
Station 3V	1969	0	1	1	1	-
	1970	0	1	4	9	-
Station 4	1969	0	0	165	118	0
	1970	0	0	3	1	0

TABLEAU 6. Valeurs de l'indice de diversité "α" de Fisher et al. (1943) pour les assemblages d'organismes se fixant sur des plaques immergées en mai et prélevées à des intervalles réguliers de 14 jours en 1969. σ_α: écart-type de l'indice α.

Station	Numéro		23 juin	7 juil.	23 juil.	4 août	18 août	30 août	13 sept.	27 sept.	11 oct.	Rang
Pointe-aux-Orignaux	1	α				0.698	0.477	0.479				5
		σ_α				0.007	0.000	0.000				
Gros-Cacouna	2	α	2.508	11.118	5.067	4.887	4.231	3.607	5.532	3.620		1
		σ_α	0.093	1.284	0.168	0.107	0.078	0.034	0.275	0.106		
Cap-Marteau	3V	α	0.751	0.732	1.985	2.158	4.260	3.096				2
		σ_α	0.010	0.000	0.003	0.004	0.008	0.009				
Cap-Marteau	3H	α	0.552	0.399	0.741	1.647	0.962	1.181	1.261	1.644	1.269	4
		σ_α	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Pointe-Métis	4	α	3.852	0.893	1.550	1.490	0.972	2.256	3.880			3
		σ_α	0.603	0.019	0.016	0.002	0.001	0.007	0.133			

Par ailleurs, à Pointe-aux-Orignaux (station 1), le nombre d'espèces est constant et faible, mais les individus sont abondants, ce qui explique les indices très bas.

En ce qui concerne les plaques immergées en juin (tableau 7), la situation est semblable à celle des plaques immergées en mai.

Discussion

Nous venons de présenter la composition, les périodes de colonisation et l'abondance saisonnière de communautés épibenthiques infralittorales de l'estuaire du Saint-Laurent, tout en soulignant au passage les influences biologiques et écologiques affectant les populations observées. En reprenant un à un les facteurs agissant sur les populations aux différentes étapes de la fixation, nous tenterons maintenant de faire ressortir plus globalement leurs effets sur la communauté épibenthique et la signification écologique de leur action.

Débuts de la communauté épibenthique

Facteurs biotiques — Nos observations sur l'intensité de la fixation de *P. ciliata* en fonction de la durée d'immersion des plaques suggèrent l'intervention d'un facteur favorisant la fixation des Polychètes sur des plaques immergées durant une longue période (1 à 2 mois). En effet, à Cap-Marteau (station 3), des plaques immergées au début de mai et prélevées au début de juillet comportent environ deux fois plus d'individus que des plaques immergées en juin et prélevées aussi au début de juillet, bien que ces deux groupes de plaques aient subi le même assaut des larves. Les expériences faites sur la phase initiale du processus de fixation ont surtout porté sur le rôle du film initial dans la fixation des larves de différents taxons (Zobell et Allen 1935; Smith et al. 1950; Wood 1950; Millard 1952; Daniel 1955; Cirino 1958; Huvé 1969). Après deux ou trois semaines d'immersion, l'existence de ce film ne fait aucun doute et nous croyons que la qualité du peuplement initial affecte la fixation de *P. ciliata*. Selon Huvé (1969), il est probable que les micro-organismes et les particules organiques initiales favorisent momentanément le développement d'une population de microphages et constituent ainsi le point de départ d'une chaîne alimentaire. A la lumière de cette hypothèse, les observations citées plus haut trouvent leur meilleure explication dans le phénomène suivant: les micro-organismes et les particules organiques du film initial sont plus abondants sur les plaques depuis longtemps immergées que sur des plaques nouvellement immergées. Les plaques à plus longue immersion sont ainsi en mesure d'héberger un plus grand nombre d'organismes — en l'occurrence *P. ciliata* — que des plaques dont le peuplement initial est encore peu abondant.

Le polychète *P. ciliata* colonise les plaques tôt en saison, alors qu'elles sont relativement vierges, ce qui n'est pas le cas des Cirripèdes. Nous avons déjà démontré (Bourget et Lacroix 1972) que pour un même assaut des larves, l'intensité de la fixation des Cirripèdes varie suivant la préparation biotique de la plaque. En bref, les résultats de ces expériences montrent: 1) qu'une période d'inhibition totale de colonisation des Cirripèdes, correspondant selon toute probabilité à la durée de la formation du film initial, se produit immédiatement après l'immersion d'une surface; 2) qu'une colonisation de plus en plus abondante d'organismes sessiles et sédentaires inhibent de plus en plus la colonisation des Cirripèdes.

Facteurs physiques — Il découle des faits exposés sur les périodes de fixation que la ponte et la fixation des organismes sont tardives dans l'estuaire du

TABLEAU 7. Valeurs de l'indice de diversité "α" pour les assemblages d'organismes se fixant sur des plaques immergées en juin et prélevées à des intervalles réguliers de 28 jours en 1969. σ_α: écart-type de l'indice α.

Station	Numéro		7 juil.	4 août	30 août	27 sept.	25 oct.	Rang
Pointe-aux-Orignaux	1	α		0.698	0.424			5
		σ_α		0.000	0.000			
Gros-Cacouna	2	α	1.056	6.086	3.705	2.967		1
		σ_α	0.007	0.229	0.201	0.104		
Cap-Marteau	3V	α	0.699	2.238	2.459			2
		σ_α	0.003	0.003	0.008			
Cap-Marteau	3H	α	0.578	1.512	1.086	1.880	0.538	4
		σ_α	0.000	0.002	0.000	0.001	0.012	
Pointe-Métis	4	α	0.954	1.792	1.466			3
		σ_α	0.080	0.003	0.003			

Saint-Laurent. La fixation intensive des larves est limitée aux mois de juin, juillet et août, en somme, lorsque la température de l'eau est relativement élevée. De plus, le retard dans la fixation de *P. ciliata* à la station de Cap-Marteau (station 3) et la densité totale plus faible en 1970 qu'en 1969 à la station de Pointe-Métis (station 4) coïncident avec un réchauffement printannier plus lent en 1970. Il est déjà connu que la ponte de plusieurs espèces — espèces pionnières sur nos plaques — telles *P. ciliata* et *B. crenatus*, est déclenchée par le réchauffement des eaux (Corlett 1948; Bousfield 1954; Dorsett 1961). Il nous semble hors de doute que le réchauffement des eaux de l'estuaire agit sur la ponte des organismes pionniers et sur la période de colonisation. D'après McDougall (1943), il est probable que les fluctuations d'abondance des organismes à vie brève sont reliées aux variations de température ou à d'autres facteurs liés indirectement aux températures.

Il ressort d'expériences précédentes (Bourget et Lacroix 1972) que sur les plaques immergées à Pointe-aux-Orignaux (station 1) durant une période de trois mois (2.5 mois avant l'assaut des larves cypris), il y a inhibition totale de la fixation des Cirripèdes, contrairement à des plaques immergées durant 2 mois (1.5 mois avant l'assaut des larves cypris) qui, elles, permettent un maximum de fixation. Nous croyons que cette inhibition est causée par une accumulation supplémentaire de sédiments (tableau 4), liée à l'extrême abondance des matières en suspension dans les parages de Pointe-aux-Orignaux (station 1).

Nous avions également discuté l'effet de la transparence de l'eau sur la qualité du film initial et sur la fixation des Cirripèdes. La turbidité de l'eau agit donc indirectement et de deux façons, sur l'installation initiale: 1) en augmentant la quantité de sédiments sur les plaques, inhibant ainsi la colonisation d'organismes pionniers (Cirripèdes); 2) en réduisant considérablement l'éclairement solaire sur les plaques, d'où un retard dans la formation du film initial et la colonisation des surfaces par les mêmes organismes.

Développement et maintien de la communauté épibenthique

Facteurs biotiques — Certaines espèces pionnières envahissent tout l'espace disponible, ce qui a pour effet de leur éviter une concurrence avec d'autres espèces. Pour y parvenir, les individus doivent se grouper densément, à un stade où leur taille est très faible. Par exemple, au début de la période de colonisation de *P. ciliata* à la station de Cap-Marteau (station 3H), la densité atteint 2661 ind./dm^2. Plus tard, la compacité du groupement sera favorisée par la position verticale des tubes. L'espèce a avantage à maintenir une forte densité, car tout en se protégeant contre les autres espèces, les individus se protègent mutuellement et résistent mieux au lessivage.

Toutefois, pour que la population parvienne à une densité élevée d'adultes, bon nombre d'individus semblent devoir être éliminés au cours de leur croissance. Nous pensons qu'une forte proportion de la diminution de la densité de *P. ciliata* à Cap-Marteau (station 3) est due à l'accroissement de la taille des individus: une telle réduction (quasi linéaire) dans les effectifs de la population agirait donc comme un phénomène dépendant de la croissance des organismes.

La densité sur les plaques immergées en mai (donc à densités plus élevées) diminue plus rapidement que sur celles immergées en juin (à densités plus faibles) à la même station (Fig. 4). Ceci suggère que la diminution très grande observée était obligatoire à cause de l'interaction inévitable entre la densité initiale élevée et la croissance.

En ce qui concerne les Cirripèdes, la compétition intraspécifique pour l'espace ne semble pas importante, puisque peu d'organismes se sont superposés, leur taille étant encore très petite.

La compétition interspécifique est maintes fois mentionnée comme important facteur de mortalité chez les Cirripèdes (McDougall 1943; Fuller 1946; Connell 1961; Cory 1967; Meadows 1969). Nos observations au sujet de la compétition interspécifique se limitent aux relations entre le Polychète *P. ciliata* et les Cirripèdes *B. balanoides* et *B. crenatus* à Cap-Marteau (station 3). *P. ciliata* s'est fixée la première et a envahi tout l'espace disponible. Quelques Cirripèdes parvinrent à se fixer, mais étant donné leur petite taille et la croissance rapide de *P. ciliata*, plusieurs d'entre eux se retrouvèrent enfouis sous les sédiments ou les tubes de Polychètes, bloquant ainsi les mécanismes de filtration. Ce phénomène de compétition entre *P. ciliata* et d'autres espèces sessiles fut également souligné par Fuller (1946), qui mentionne que la masse de tubes et de sédiments couvre totalement certaines plaques et élimine toutes les espèces à l'exception des Moules communes.

Facteurs physiques — La turbidité de l'eau, directement, ou indirectement par la quantité de sédiments déposée sur les plaques, agit sur la fixation de plusieurs groupes d'organismes: nous avons vu qu'à Pointe-aux-Orignaux (station 1) elle agit sur les Cirripèdes au moment de l'installation de départ. Toutefois, à certaines stations d'étude (Cap-Marteau, station 3), les Cirripèdes ne sont pas pionniers, mais doivent s'installer entre les orga-

ganismes déjà bien établis. Il n'en reste pas moins que les sédiments agissent tout autant, et de la même manière, sur la fixation des larves cypris.

En ce qui concerne l'action des sédiments sur le maintien des formes sessiles, le tableau 5 montre la tendance qu'ont les individus (à Pointe-aux-Orignaux en particulier, par suite de l'extrême abondance des matières en suspension) à être enfouis progressivement sous les sédiments. Nos données de Pointe-aux-Orignaux (station 1) sont recueillies sur une trop courte période pour conclure à l'importance de l'accumulation des sédiments comme facteur de mortalité. Sur une longue période (2 ou 3 mois) nous croyons toutefois que ce facteur (le seul identifié à Pointe-aux-Orignaux) peut jouer un rôle important. D'ailleurs, McDougall (1943) mentionne l'action néfaste des sédiments sur les Cirripèdes.

Bien que le mouvement des eaux puisse agir pour favoriser la fixation de certaines larves de Cirripèdes (Balanes) ou même accélérer le processus de croissance d'organismes sessiles en renouvellant continuellement les stocks de nourriture, il arrive que les vagues (particulièrement à faible profondeur) éliminent certaines espèces en les arrachant.

Lorsque le nombre d'individus diminue, on doit espérer atteindre un équilibre compétitif entre les individus, et la population devrait présenter une certaine stabilité. Or, l'analyse des résultats révèle que malgré l'absence de fixation importante et un nombre d'individus relativement faible, presque toutes les populations décroissent continuellement, certaines jusqu'à une élimination complète durant l'automne. Dans le cas de quelques autres — bien que nous ayons pu constater leur acheminement vers une extinction — elles ne furent pas suivies assez longtemps pour qu'on constate une disparition. Certains dommages observés sur les plaques à la suite de tempêtes automnales, tels que l'arrachement d'algues, le lessivage de tubes de Polychètes, suggèrent que l'action des tempêtes automnales est des plus importantes puisqu'elle agit sur la partie résiduelle de la population, celle-là même qui doit assurer le repeuplement.

Contrôle de la communauté épibenthique

Toutes les communautés sont modelées par l'action de leurs composantes physiques et biologiques, et suivant la proportion relative des unes et des autres, la structure de la communauté s'en trouve modifiée. Il n'y a pas de communauté sous le contrôle exclusif des facteurs physiques, pas plus qu'il n'y a de communauté sous le contrôle exclusif de facteurs biotiques (Sanders 1968). Le contrôle de la communauté épibenthique infralittorale de l'estuaire du Saint-Laurent diffère suivant les saisons. Cette communauté épibenthique est biologiquement plus harmonisée en été, étant donné la très grande compétition intraspécifique et interspécifique pour l'espace. Par ailleurs, le contrôle par les facteurs physiques est prédominant en automne, lorsque les tempêtes automnales agissent sur des densités déjà faibles, et en hiver, par l'action abrasive des glaces.

Ce mode de contrôle saisonnier empêche les populations d'atteindre un équilibre compétitif ou même de s'éliminer entre elles. Ceci se répercute sur les phénomènes de succession de peuplement. Quelques cas de vraies successions dans les communautés animales du milieu marin furent mis en évidence (Hewatt 1935; Sheer 1945; Huvé 1953; Cirino 1958). Il est possible qu'en milieu tropical ou tempéré on puisse noter des phénomènes de succession; cependant, en milieu sub-arctique, où les saisons accentuées agissent pour comprimer les phénomènes de fixation en plus d'agir considérablement sur les conditions physiques des eaux littorales, il est peu probable que de véritables successions épibenthiques se produisent. Dans l'estuaire du Saint-Laurent, nous n'avons pas noté de successions de peuplement: comme nous venons de le souligner les interactions biotiques jouent un rôle très secondaire dans l'élaboration du peuplement semi-définitif automnal.

Enfin, comme le souligne Sanders (1968), lorsqu'il y a un contrôle physique prédominant sur une communauté, il ne peut y avoir une relation étroite entre l'espèce et son milieu comme dans une communauté biologiquement harmonisée. C'est à dire qu'il n'y a jamais une réitération exacte d'une année à l'autre. Ce point est bien mis en évidence par l'abondance des individus en 1969 et la grande pauvreté en 1970.

De l'analyse des faits, il ressort que la communauté épibenthique de l'estuaire du Saint-Laurent est biologiquement harmonisée pendant l'été, et plus physiquement contrôlée durant la période automne-hiver. Cependant, il convient de mentionner que la colonisation et la destruction des communautés est amplifiée sur des surfaces artificielles uniformes. Dans les milieux naturels, la destruction n'est pas aussi catastrophique que sur les plaques, et la survie d'un bon nombre de reproducteurs est assurée en des endroits abrités où le morcellement des surfaces fournit une protection plus grande aux individus.

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BOURGET, E., AND G. LACROIX. 1971. Two simple durable epifaunal collectors. J. Fish. Res. Board Can. 28: 1205–1207.

1972. Colonisation et inhibition de la colonisation des Cirripèdes dans l'estuaire du Saint-Laurent. Natur. Can. 99: 279–285.

BOUSFIELD, E. L. 1954. The distribution and spawning seasons of barnacles on the Atlantic Coast of Canada. Bull. Nat. Mus. Can. 132: 112–154.

CIRINO, E. F. 1958. Evidence of true succession in marine littoral associations. Diss. Abstr. 19(6): 174 p.

CONNELL, J. H. 1961. The influence of interspecific competition and other factors on the distribution of the Barnacle *Chthamalus stellatus*. Ecology 42: 719–723.

CORLETT, J. 1948. Rate of settlement and growth of the "pile" fauna of the Mersey Estuary. Proc. Liverp. Biol. Soc. 52: 2–28.

CORY, R. L. 1967. Epifauna of the Patuxent River Estuary, Maryland, for 1963 and 1964. Chesapeake Sci. 8: 71–89.

DANIEL, A. 1955. The primary film as a factor in settlement of marine foulers. J. Madras Univ. 25B(2): 189–200.

DIONNE, J. C. 1968. Action of shore ice on the tidal flats of the St. Lawrence Estuary. Mar. Sediments 4: 113–115.

DORSETT, D. A. 1961. The reproduction and maintenance of *Polydora ciliata* (Johnst.) at Whitstable. J. Mar. Biol. Ass. U.K. 41: 383–396.

FISHER, R. A., A. S. CORBETT, AND C. B. WILLIAMS. 1943. The relation between the number of species and the number of individuals in a random sample of an animal population. J. Anim. Ecol. 12: 42–58.

FULLER, J. L. 1946. Season of attachment and growth of sedentary marine organisms at Lamoine, Maine. Ecology 27: 150–158.

GAUDRY, R. 1938. Les températures de l'estuaire du Saint-Laurent. Contrib. Sta. Biol. St-Laurent, Univ. Laval 13: 1–14.

HEWATT, W. G. 1935. Ecological succession in the *Mytilus californianus* habitat as observed in Monterey Bay, California. Ecology 16: 244–251.

HOPKINS, A. E. 1935. Attachment of larvae of the Olympia Oyster, *Ostrea lurida*, to plane surfaces. Ecology 16: 82–87.

HUVÉ, P. 1953. Compte-rendu préliminaire d'une expérience de peuplement de surfaces immergées. Rec. Trav. Sta. Mar. Endoume, Fac. Sci. Marseille 8 (Bull. 3).

1969. Le stade initial et son importance réelle dans le peuplement algal des surfaces rocheuses du littoral marin. Proc. Int. Seaweed Symp. 6: 201–211.

MCDOUGALL, K. D. 1943. Sessile marine invertebrates at Beaufort, North Carolina. Ecol. Monogr. 13: 321–374.

MEADOWS, P. S. 1969. Sublittoral fouling communities on Northern Coast of Britain. Hydrobiologia 34: 273–294.

MILLARD, N. 1952. Observ ns and experiments on fouling organisms in Table Bay Harbour, South Africa. Trans. Roy. Soc. S. Afr. 33: 415–446.

MOORE, H. B. 1935. The biology of *Balanus balanoides*. IV. Relation to environmental factors. J. Mar. Biol. Ass. U.K. 20: 277–307.

NADEAU, A. 1938. Salinité des eaux de l'estuaire du St-Laurent. Contrib. Sta. Biol. St-Laurent, Univ. Laval 11: 1–19.

NOTA, D. J. G., AND D. H. LORING. 1964. Recent depositional conditions in the St. Lawrence River and Gulf–A reconnaissance survey. Mar. Geol. 2: 198–235.

PARDÉ, M. 1948. Hydrologie du Saint-Laurent et de ses affluents. Rev. Géogr. Montréal 2: 35–84.

POMERAT, C. M., AND E. R. REINER. 1942. The influence of surface angle and of light in the attachment of barnacles and of other sedentary organisms. Biol. Bull. 82: 14–25.

RUNNSTRÖM, S. 1925. Zur Biologie und Entwicklung von *Balanus balanoides* (Linné). Bergens Mus. Arb. 1924–25, Naturv. Rekke 5: 46 p.

SANDERS, H. L. 1968. Marine benthic diversity: a comparative study. Amer. Natur. 102: 243–282.

SENTZ-BRACONNOT, E. 1966. Données écologiques sur la fixation d'invertébrés sur des plaques immergées dans la rade de Villefranche-sur-mer. Int. Rev. Ges. Hydrobiol. 51: 461–484.

SHEER, B. T. 1945. The development of marine fouling communities. Biol. Bull. 89: 103–121.

SMITH, F. G. W., R. H. WILLIAMS, AND C. C. DAVIS. 1950. An ecological survey of the subtropical inshore waters adjacent to Miami. Ecology 31: 119–146.

SYMON-PAPYN, L. 1965. Installation expérimentale du benthos sessile des petits substrats durs de l'étage circalittoral en Méditerranée. Rec. Trav. Sta. Mar. Endoume Fac. Sci. Marseille 39(55): 53–94.

VISSCHER, J. P. 1928. Reactions of the cyprid larvae of barnacles at the time of attachment. Biol. Bull. 54: 327–335.

VISSCHER, J. P., AND R. H. LUCE. 1928. Reactions to the cyprid larvae of barnacles to light, with special reference to spectral colors. Biol. Bull. 54: 336–350.

WILLIAMS, C. P. 1964. Patterns in the balance of nature. Academic Press, London. 324 p.

WOOD, E. J. F. 1950. Investigations on underwater fouling I. The role of bacteria in the early stages of fouling. Aust. J. Mar. Freshwater Res. 1: 85–91.

ZOBELL, C. E., AND E. C. ALLEN. 1935. The significance of marine bacteria in the fouling of submerged surfaces. J. Bacteriol. 29: 239–251.

Life Cycle of *Salmincola californiensis* (Dana 1852) (Copepoda: Lernaeopodidae)

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KABATA, Z., AND B. COUSENS. 1973. Life cycle of *Salmincola californiensis* (Dana 1852) (Copepoda: Lernaeopodidae). J. Fish. Res. Board Can. 30: 881–903.

A description is given of the life cycle of *Salmincola californiensis* (Copepoda: Lernaeopodidae), studied by continuous observation of developing parasites and by intermittent examination of infected sockeye salmon (*Oncorhynchus nerka*). The cycle consists of six stages: copepodid (free-swimming), four chalimus stages (attached by second maxillae to frontal filament), and adult (attached by bulla). The copepodid settles on the fish and uncoils its frontal filament. Each successive chalimus stage reattaches the filament during moulting, but chalimus IV breaks off, searches for the site of final attachment, and excavates an implantation cavity for the bulla. The male cycle is similar but its chalimus IV is of shorter duration and soon moults into the male adult, actively searching for the female. Morphology of all stages is described in detail. Some comments are made on life cycles in Caligoida.

KABATA Z., AND B. COUSENS. 1973. Life cycle of *Salmincola californiensis* (Dana 1852) (Copepoda: Lernaeopodidae) J. Fish. Res. Board Can. 30: 881–903.

La description qui suit du cycle biologique de *Salmincola californiensis* (Copepoda: Lernaeopodidae) est basée sur des observations continues de parasites en voie de développement et l'examen périodique d'*Oncorhynchus nerka* infestés. Le cycle comprend six stades: un copépodite (libre), quatre chalimus (fixés au filament frontal par les maxilles) et l'adulte (fixé par la bulle). Le copépodite se fixe sur l'hôte et déroule son filament frontal. Chaque stade chalimus qui suit fixe de nouveau le filament durant la mue, mais le chalimus IV se détache, cherche un site définitif de fixation, et creuse une cavité pour l'implantation de la bulle. Le cycle biologique du mâle est semblable, mais le stade chalimus IV dure moins longtemps et se transforme rapidement en adulte qui se met activement à la recherche d'une femelle. Nous décrivons en détail la morphologie de tous les stades, et faisons quelques commentaires sur les types de cycle biologique rencontrés chez les Caligoida.

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IN view of their association with economically important fishes, the parasitic copepods of the genus *Salmincola* have attracted a good deal of attention. In spite of the fact that abundant literature has accumulated on the subject of *Salmincola*, our knowledge of this genus of potentially harmful copepods is far from satisfactory. Until recently, even the taxonomy of *Salmincola* was in a state of confusion.

Present trends towards large-scale development of fish farming bring with them increased dangers of parasitic depredations, to which dense populations of cultured fish are particularly vulnerable. Elaboration of protective or therapeutic measures, effective against parasites that are likely to become troublesome under those conditions, must be developed in time to meet the needs of fish culturists. The development of such methods is possible only when one becomes intimately acquainted with the biology of the parasite in question, at all stages of its life cycle.

So far, the life cycle of *Salmincola*, though studied much more extensively than those of most other copepod parasites, cannot be considered as sufficiently well known. Zandt (1935) published an account

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of the postembryonal development of *Salmincola coregonorum* (Kessler 1868). Dedie (1940) studied the life history of *S. edwardsii* (Olsson 1869) and Friend (1941) that of *S. salmoneus* (L.). A series of publications by Fasten (1913, 1914, 1919, 1921) dealt with various aspects of biology of *Salmincola* living on the salmonids in the western part of North America. All these publications gave us a general appreciation of the pattern of life cycles within the genus *Salmincola*.

Following the taxonomic revision of *Salmincola* (cf. Kabata 1969), it became clear that all species of the Pacific salmon and trout are parasitized by only one species of this genus, *S. californiensis* (Dana 1852). This paper describes its life cycle and morphology of individual stages.

Materials and Methods

Previous investigators dealing with the life cycle of *Salmincola* used one of two methods: they either studied the life cycle in the laboratory, by following it, moult by moult, under a microscope (Zandt 1935), or examined commercially-caught fish and collected from them what they considered to be a complete series of developmental stages. Both methods suffer from certain disadvantages. The former involves introduction of artificial conditions, such as high intensity illumination and abnormally high temperature, prevailing during microscopic observations. Under those conditions the developmental processes are subject to distortions, the extent of which is difficult to assess. The latter method has in it an inherent danger of missing one or several stages, as evident in the work of Friend (1941).

We decided to track the progress of development of *S. californiensis* by using modifications of both these methods, carried out completely under laboratory conditions. The first of them, the method of almost continuous observation, permitted us to piece together the entire life cycle, stage by stage. The egg-sacs (if possible with eggs at pigmented stage) were collected from adult females, placed in Petri dishes with tap water, and kept in a refrigerated shaker bath at 10 C. Free-swimming copepodids that hatched from these eggs were transferred to 500-ml beakers containing sockeye salmon (*Oncorhynchus nerka*), fork length between 3 and 4 cm. The size of the beakers made it possible to observe their contents under a dissecting microscope. Settling of copepodids, their subsequent moults, and progress of ontogeny were followed by direct observation. The second method consisted of infecting *O. nerka*, kept in aquaria in batches of 50 fish, with copepodids hatched out as in previous experiments. The size of fish was also comparable with that of fish used earlier. The stage of development attained in a given time was determined by sacrificing several fish at set intervals and examining attached parasites. Examinations were carried out at least twice daily. The method permitted a fairly accurate timing of ontogenetic events.

In view of difficulties experienced by workers trying to rear parasitic copepods from eggs, it is worth noting that *Salmincola* was hatched be resorting to Petri dishes filled with water, unfiltered and without addition of antibiotics. The proportion of failures was negligible and was mainly due to fungal infections. Water in Petri dishes was changed at infrequent intervals or not at all.

Morphology of Developmental Stages

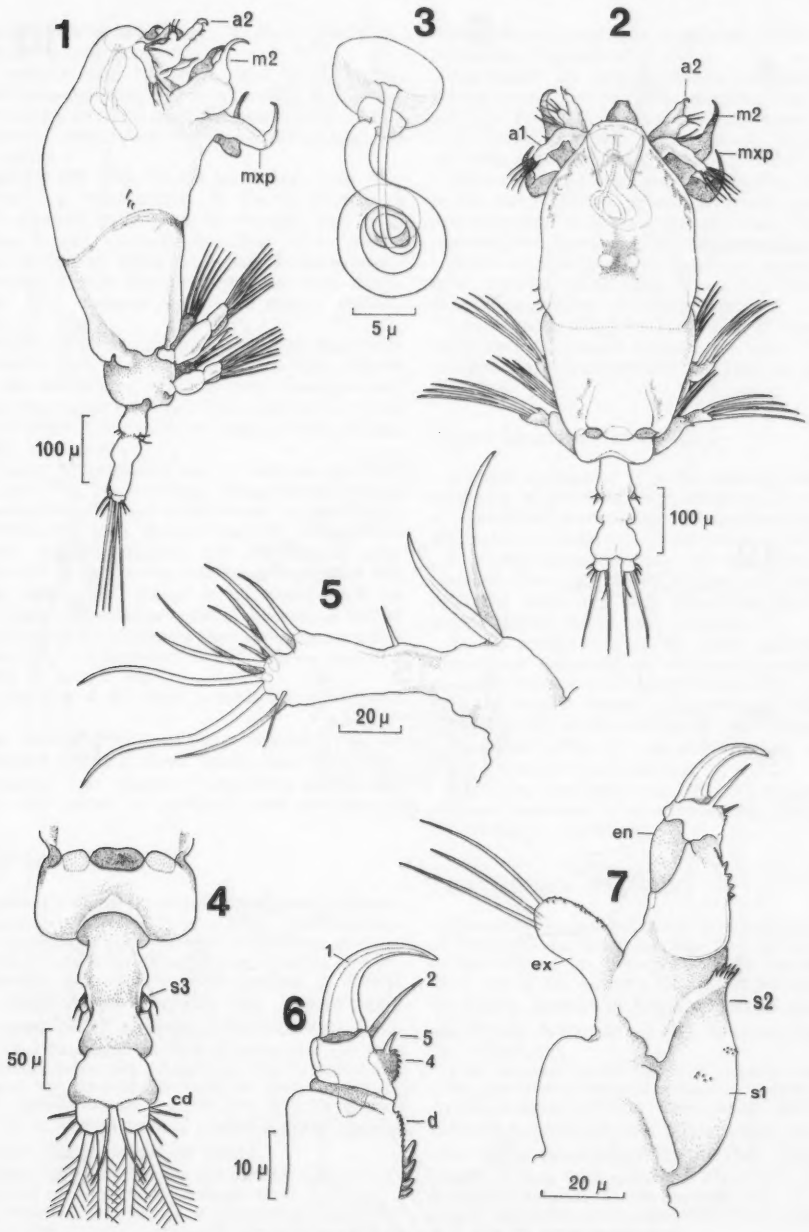
COPEPODID (FIG. 1–16)

Forebody anteriorly fusiform, posteriorly slightly depressed (Fig. 1), oval in dorsal view (Fig. 2), with indistinct transverse constriction at about midlength. Anterior margin rounded, armed with six minute sensory setules. Median eye near center of forebody on dorsal surface, frontal filament between it and anterior margin (Fig. 2). Filament (Fig. 3) in three loops, thick at base, tapering distally, with large plug at proximal and smaller at distal end. Lateral margins of forebody nearly parallel, armed with three setules on either side directly anterior to transverse constriction. Posterior margin (Fig. 4) with central notch and one lateral process on either side, both overlapped by similar processes of hindbody. First and second antennae, buccal apparatus (including two pairs of maxillae), and maxillipeds on ventral side of anterior part of forebody. First pair of swimming legs on ventrolateral part of posterior end of forebody, second pair on ventral surface of hindbody. Latter (Fig. 4) of two parts: broad and short anterior (about one-half width of forebody in its widest part) with rounded posterolateral corners, and narrow posterior of three indistinctly delimited segments. First segment with vestigial third legs of lateral margins just posterior to prominent marginal outgrowths. Second (last thoracic) segment shorter than first, expanding posteriorly. Third segment (pseudoabdomen) also posteriorly broader and slightly longer than second, with caudal rami on posterior margin. Average length (in μ based on 10 specimens) 690 (660–720).

First antenna (Fig. 5) short and not distinctly segmented, with base on anterior margin of forebody, slightly lateral to large plug of frontal filament. Basal part short and stout, imperceptibly passing into narrower, clavate distal part. Two very long, flat, curving setae on anterior margin of basal part. Third, short, and straight seta near centre of appendage. Two more setae on subterminal swelling of anterior margin and nine more apical setae, two short, others long or moderately long.

Second antenna (Fig. 6–8) biramous. Sympod (Fig. 7, s1, s2) indistinctly two-segmented, armed with three batches of spinules on ventral wall. Distalmost group of setae much larger than other two, arranged in single row (Fig. 8). Endopod (Fig. 6, 7, en) two-segmented, with row of prominent denticles and another of smaller denticles (Fig. 6, d) on ventral surface of basal segment. Apical armature as in other Lernaeopodidae; powerful hook 1 with spiniform seta 2 on medial side of its base, inflated ventral wall 4 with row of denticles and setiform process 5. (No trace of tubercle 3, commonly present in Lernaeopodidae, was found on lateral side of base of hook 1.)

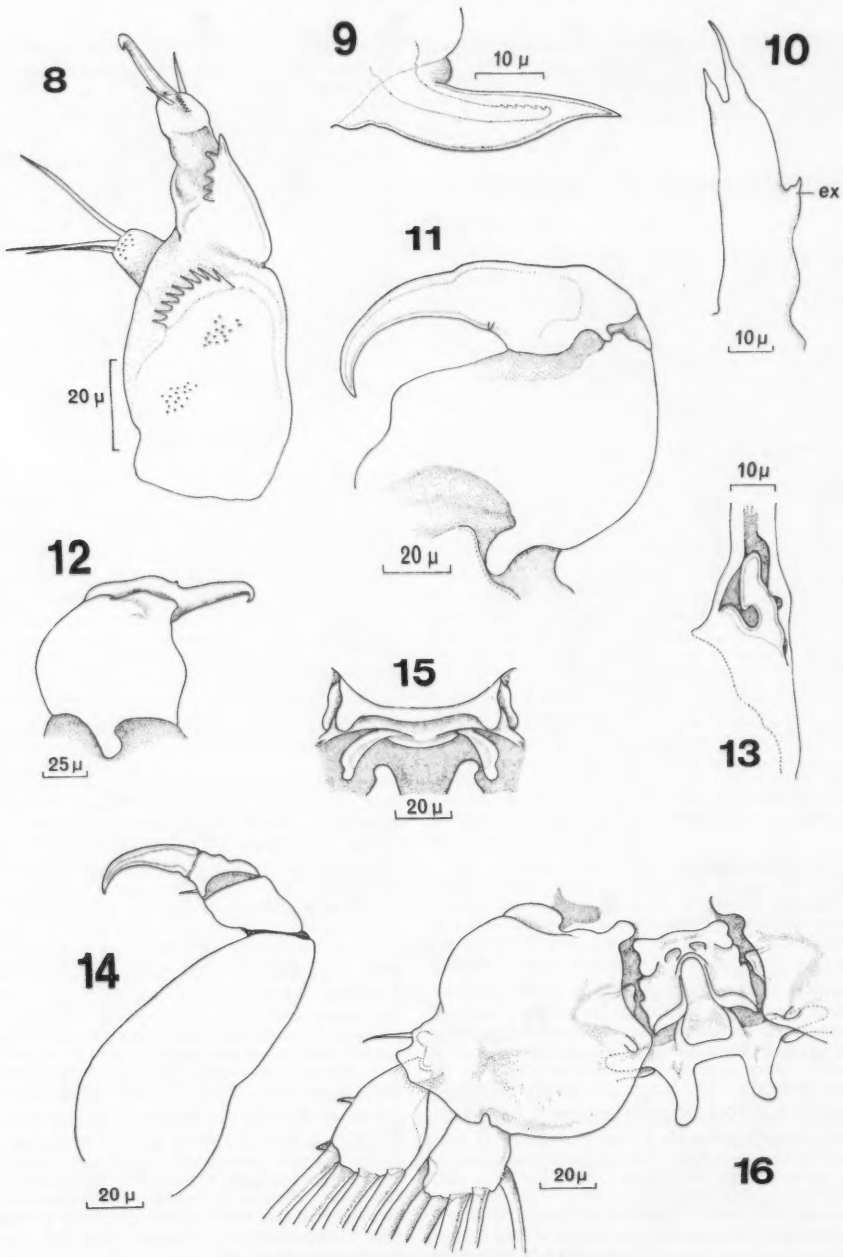
Mouth tube with upper (smaller) and lower (larger) lip, partially fused and similar to those of all other



FIGURES 1–7. *Salmincola californiensis*, copepodid.

FIG. 1. Entire, lateral; FIG. 2. Same, dorsal; FIG. 3. Coiled frontal filament, ventral; FIG. 4. Posterior part of body, dorsal; FIG. 5. First antenna, dorsal; FIG. 6. Second antenna, tip of endopod, lateral; FIG. 7. Same, entire, medial.

[a1, first antenna; a2, second antenna; cd, caudal ramus; d, denticles; en, endopod; ex, exopod; m2, second maxilla; mxp, maxilliped; s1, first segment of sympod; s2, second segment of sympod; s3, vestige of third leg; other explanations in text]



FIGURES 8–16. *S. californiensis*, copepodid.

FIG. 8. Second antenna, entire, ventral; FIG. 9. Mandible, lateral; FIG. 10. First maxilla, ventrolateral; FIG. 11. Second maxilla, corpus ventral, claw lateral; FIG. 12. Same, ventral, in situ; FIG. 13. Same, base of claw, medial; FIG. 14. Maxilliped, ventral; FIG. 15. First interpodal bar, ventral (legs pointing forward); FIG. 16. Second leg and interpodal bar, ventral (legs pointing backward).

[ex, exopod]

lernaeopodids. Mandible (Fig. 9) short, blade-like, with sharp tip and no teeth.

First maxilla (Fig. 10) short, with small, lateral, tubercle-like exopod (ex) tipped with short seta. Distal end of endopod with two papillae, imperceptibly passing into terminal setae, short on ventral and longer on dorsal papilla.

Second maxilla (Fig. 11–13) subchelate, with one-segmented, flat, broad corpus. In Fig. 11 position of subchela distorted by pressure of coverslip. In Fig. 12 appendage in situ. Cuticular flap (Fig. 13) at base of subchela serving as guide controlling its movements. Subchela not divided into shaft and claw, with stubby seta near base, abruptly tapering to sharply hooked, fine tip.

Maxilliped (Fig. 14) more slender and apparently less powerful than second maxilla, with long, narrow corpus and subchela of three segments (two-segmented shaft and one-segmented claw). Fine, short seta on inner margin of shaft. Claw about as long as shaft, slender, with fine, curving tip.

Two pairs of swimming legs of similar structure. Second pair (Fig. 16) biramous. Unsegmented sympod with short seta near base of exopod. Rami one-segmented. Exopod with four long, pinnate setae and two denticles on lateral margin, endopod with six pinnate setae. Elaborate set of interlocking sclerites as interpodal bar. First leg differs from second in additional setule on lateral margin of exopod near distal denticle, as well as by possession of seven pinnate setae on endopod. Interpodal bar (Fig. 15) narrower and of different structure than that of second leg.

Third leg (Fig. 4, s3) small tubercle tipped with two setae.

Caudal ramus (Fig. 4, cd) quadrangular, with six setae. Medial seta and three lateral setae short, fine, and unarmed. Two posterior setae long and robust, pinnate, with pinnae of adjacent setae overlapping.

First Chalimus (Fig. 17–25)

No distinct division between forebody and hindbody, or between two parts of former (Fig. 17). No dorsoventral flattening in posterior part of forebody. No segmentation of hindbody. All appendages heavier, shorter, with reduced armature. Conical swelling in frontal region, dorsal to base of mouth cone. Average length (in μ, based on 12 specimens) 706 (660–740).

First antenna similar to that of copepodid but with much reduced setae. Second antenna (Fig. 19) essentially unchanged but divisions between its parts no longer definite. Spinulation of sympod less well developed. Process 5 on ventral wall of exopod's distal segment either very difficult to see or absent.

Mandible (Fig. 20) flat, its dentiferous margin with series of six or seven fairly uniform teeth.

First maxilla (Fig. 21) as in preceding stage, but with third, short, apical papilla, about as large as exopod.

Second maxilla (Fig. 22) with greatly reduced subchela represented by short claw with recurved two-pronged bar, also present in subsequent stages (Fig. 30). Bars embedded in basal plug of frontal filament with some adhesive substance coagulated round point of penetration (Fig. 18, ad).

Maxilliped on ventral surface of cephalothorax behind second maxilla, rather shapeless (Fig. 23), with subchela not clearly delimited and almost straight. Claw reduced, slender, and relatively short, though still with small seta near base.

Swimming legs of both pairs similar (Fig. 24), reduced in size and simplified, without interpodal bar. Sympod one-segmented, both rami two-segmented. First exopod segment with short setae in distolateral corner, distal segments of both rami with two short, relatively strong setae, fine-tipped and much shorter than corresponding setae of copepodid. Third leg absent.

Caudal ramus (Fig. 25) tubercle-like, with another and much smaller, conical tubercle on apex. One short, unarmed seta on distomedial and three on lateral side of ramus.

Second Chalimus (Fig. 26–32)

General appearance (Fig. 26) differing only slightly from that of preceding stage. More pronounced fusion of tagmata and greater reduction of appendages. Average length (in μ, based on 10 specimens) 714 (680-760).

First and second antenna and mandible as in first chalimus. First maxilla (Fig. 28) also resembling that of preceding stage, but with dorsalmost third terminal papilla slightly larger than previously.

Second maxilla (Fig. 29, 30) longer and more robust than that of first chalimus, with broadly expanded base gradually merging with cephalothorax. Duct orifice near base, on medial margin of appendage (Fig. 29, d). Claw (Fig. 30) similar to that of first chalimus.

Maxilliped (Fig. 31) slender, tapering, with claw shorter than in first chalimus.

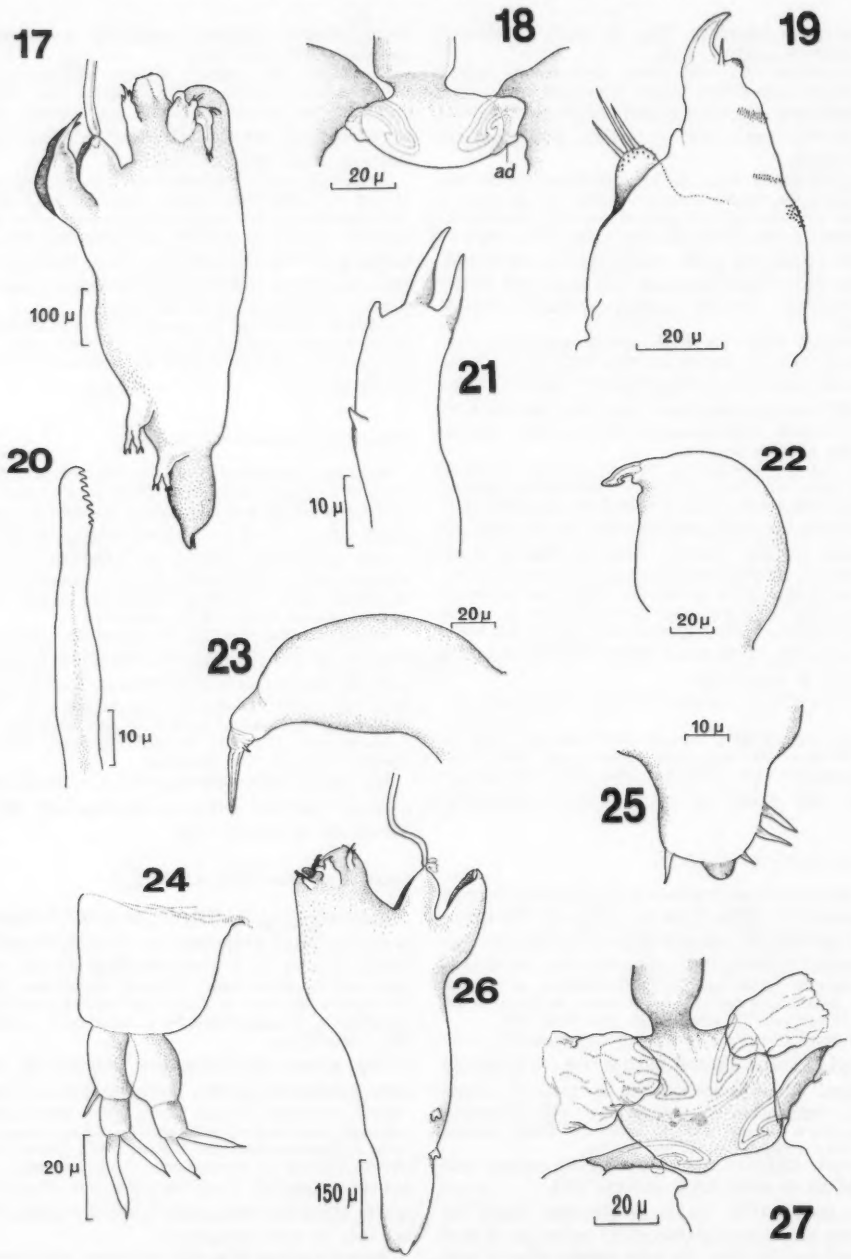
Two pairs of swimming legs (Fig. 32) reduced to pairs of small, unarmed lobes surmounting light swellings. No change in caudal rami.

Third Chalimus (Fig. 33–41)

Hindbody elongated (Fig. 33), as evident from position of much-reduced swimming legs. First leg situated near middle of body (in first chalimus near border between third and fourth quarter). General appearance affected by relative increase in length of second maxillae and maxillipeds. Average length (in μ, based on 12 specimens) 792 (760–820).

First antenna digitiform, with rounded tip and (in some specimens) apparent transverse division (Fig. 35). Apical armature of four short, blunt setae and one tubercle, homologous with corresponding structures of other Lernaeopodidae (cf. Kabata 1963, 1964a). Recognizable among its components digitiform seta 4, with associated tubercle 1; on opposite side of apex long seta 6; apical two setae, presumably of complex 5 (only one seta in some specimens).

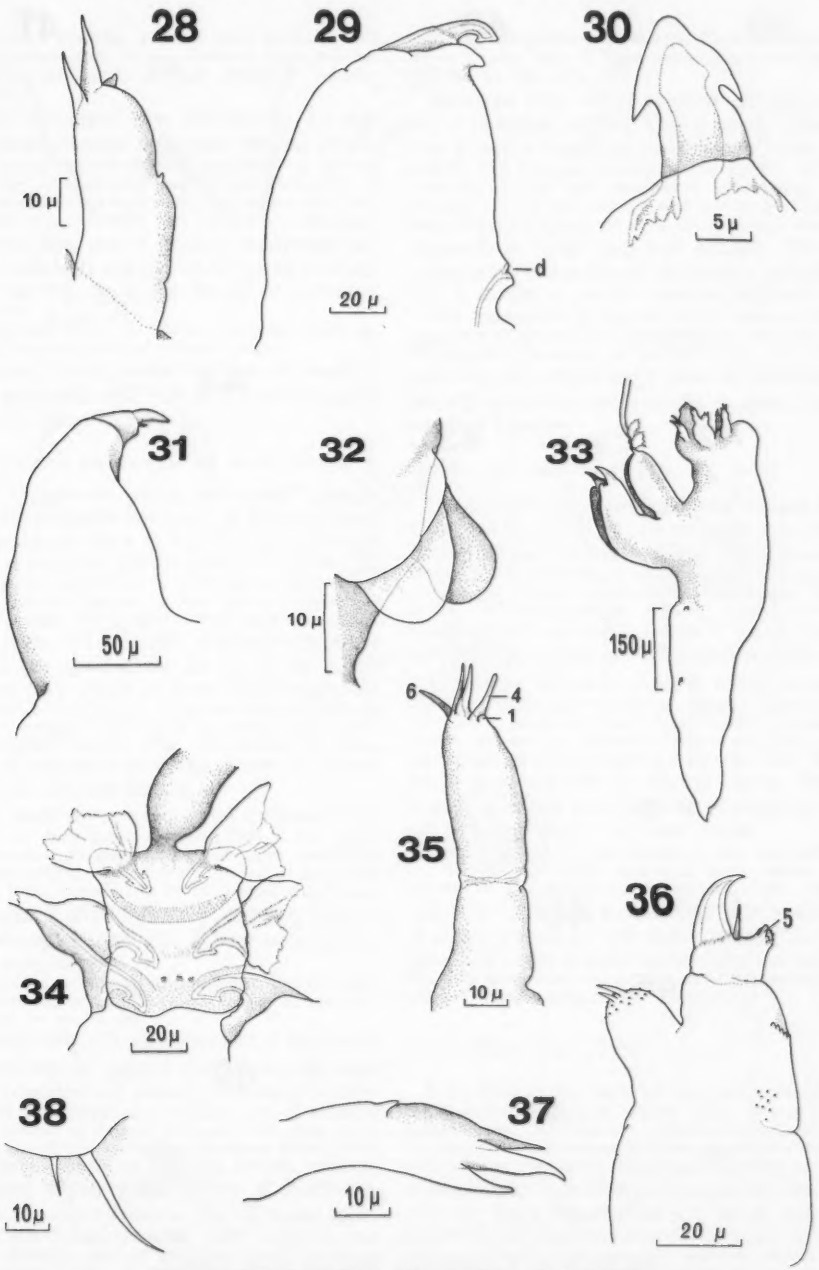
Second antenna (Fig. 36) somewhat resembling that of the adult in details. Apex of exopod with only two short processes, in addition to sparse spinulation. Ventral wall of second segment of endopod developed



FIGURES 17–27. *S. californiensis*, first and second chalimus.

FIG. 17. First chalimus, lateral; FIG. 18. Base of frontal filament and tips of second maxillae; FIG. 19. Second antenna, medial; FIG. 20. Mandible, lateral; FIG. 21. First maxilla, lateral; FIG.22. Second maxilla, ventral; FIG. 23. Maxilliped, ventral; FIG. 24. First leg, ventral; FIG. 25. Caudal ramus, ventral; FIG. 26. Second chalimus, lateral; FIG. 27. Base of frontal filament and tips of second maxillae.

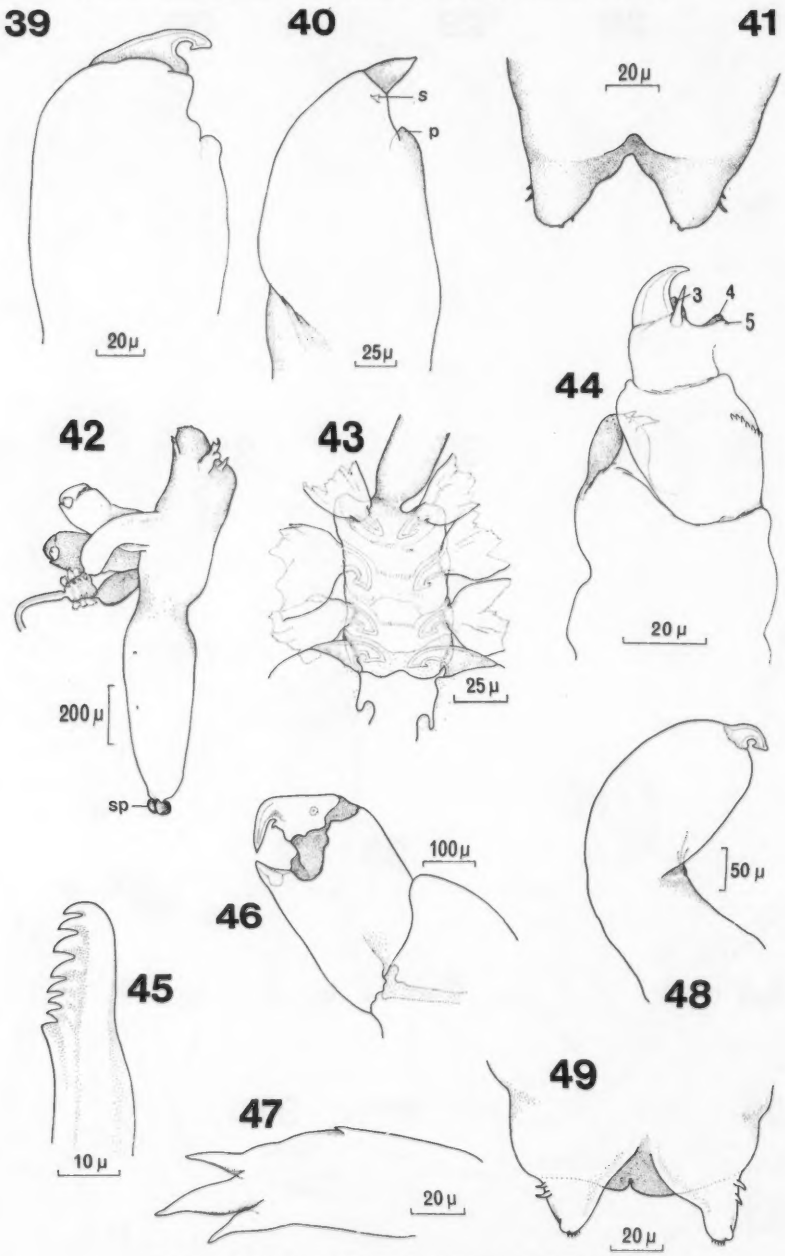
[ad, adhesive substance]



FIGURES 28–38. *S. californiensis*, second and third chalimus.

FIG. 28. Second chalimus, first maxilla, ventrolateral; FIG. 29. Second maxilla, ventral; FIG. 30. Same, tip of claw, anterior; FIG. 31. Maxilliped, anterior; FIG. 32. Second leg, dorsolateral; FIG. 33. Third chalimus, lateral; FIG. 34. Base of frontal filament and tips of second maxillae; FIG. 35. First antenna, medial; FIG. 36. Second antenna, medial; FIG. 37. First maxilla, ventrolateral; FIG. 38. Second leg, lateral.

[d, duct orifice; other explanations in text]



FIGURES 39–49. *S. californiensis*, third and fourth chalimus.

FIG. 39. Third chalimus, second maxilla, anterior; FIG. 40. Maxilliped, anterior; FIG. 41. Caudal furca, ventral; FIG. 42. Early fourth chalimus, female, lateral; FIG. 43. Fourth chalimus male, base of frontal filament and tips of second maxillae; FIG. 44. Female and male, second antenna, medial; FIG. 45. Mandible, lateral; FIG. 46. Female, maxilliped, ventral; Fig. 47. Male and female, first maxilla, ventrolateral; FIG. 48. Female, second maxilla, anterior; FIG. 49. Male and female, caudal furca, ventral.

[p, palp; s, basal seta; sp, spermatophores; other explanations in text]

into prominent swelling, covered with denticles and with short process 5 on its side. Terminal hook shorter, more robust, particularly at base, though it remains very mobile.

Mandible unchanged. First maxilla (Fig. 37) with relatively more elongate dorsalmost terminal papilla, now as long as ventral. Second maxilla (Fig. 39) resembling that of preceding stage. Claw embedded in second drop added to bottom of frontal filament (Fig. 34).

Maxilliped (Fig. 40) with claw reduced to elongated cone at apex. Basal part of subchela incorporated into appendage, with basal seta (Fig. 40, s) on wall of corpus. Conical outgrowth on medial margin of appendage (Fig. 40, p).

Swimming legs (Fig. 38) barely noticeable swellings, surmounted by two setae of unequal length.

Caudal rami (Fig. 41) resembling those of preceding stage, but apparently with only one, relatively shorter seta on each lateral margin.

Fourth Chalimus Female (Fig. 42, 44–49, 65–67, 73)

Unlike in previous stages, considerable changes occurring due to growth throughout its duration. Newly moulted female as shown in Fig. 42. Development of waist-like constriction behind bases of maxillipeds, suggesting future subdivision of body into cephalothorax and trunk. Cephalothorax at first about as long as, or slightly longer than, trunk. Both tagmata in line with each other. With progress of growth appearance changed to that shown in Fig. 66 and 67. By end of chalimus IV stage length of trunk constituting about three-quarters of total length. Long axis of cephalothorax now tilting ventrally from that of trunk. Some early fourth chalimus females (Fig. 42) already carrying remnants of spermatophores (sp.), present in almost all late fourth chalimus females.

Average length (in μ, based on 10 specimens) 1016 (960–1100) for early stage, reaching 2500 at late stage.

First antenna resembling that of third chalimus. Second antenna (Fig. 44) also similar to that of preceding stage, with fewer denticles on tip of exopod and one on ventral process 4, distal segment of endopod. Relative sizes of processes 4 and 5 altered, both processes now of about same length.

Mandible (Fig. 45) almost identical with that of adult, though rather narrower. Dentiferous margin armed with series of six or seven teeth.

First maxilla (Fig. 47) resembling that of third chalimus. Second maxilla changing shape during this stage, becoming cylindrical and gradually increasing in length (Fig. 48), its tips embedded in another drop of secretion added to bottom of frontal filament, with three pairs of empty claw cuticle identifying chalimus stage. (Male fourth chalimus shown in Fig. 43.) Second maxillae, at first linked in this manner in front of maxillipeds, later move to position shown in Fig. 42, behind maxillipeds. At late fourth chalimus, when female is free of frontal filament, second maxillae usually occur in condition shown in Fig. 73. Remnant of frontal filament (maxillary bridge) still linking their tips. In some specimens (Fig. 66, 67) bridge missing, second maxillae executing independent movements. Terminal claws, often rather smaller than in those remaining embedded, still retained at tips (Fig. 65).

Maxilliped (Fig. 46) considerably changed, arising out of prominent swelling. Corpus heavily sclerotized, with triangular outgrowth in distomedial corner. Outgrowth with concave surface for reception of tip of subchela. Latter not subdivided, resembling short, strongly curved claw, with broad and strong base and sharp, tapering tip. At point of flexure, short dentiform outgrowth on inner margin of subchela. (We were unable to find seta on base of this structure, but indistinct spot is present in position normally occupied by it.)

First leg similar to that of third chalimus, second consisting of single seta. At late fourth chalimus stage legs usually impossible to find, probably shed. Caudal rami (Fig. 49) without single setae on medial margins but with groups of minute setules at apices, close to subapical tubercles.

Fourth Chalimus Male (Fig. 43, 50–52)

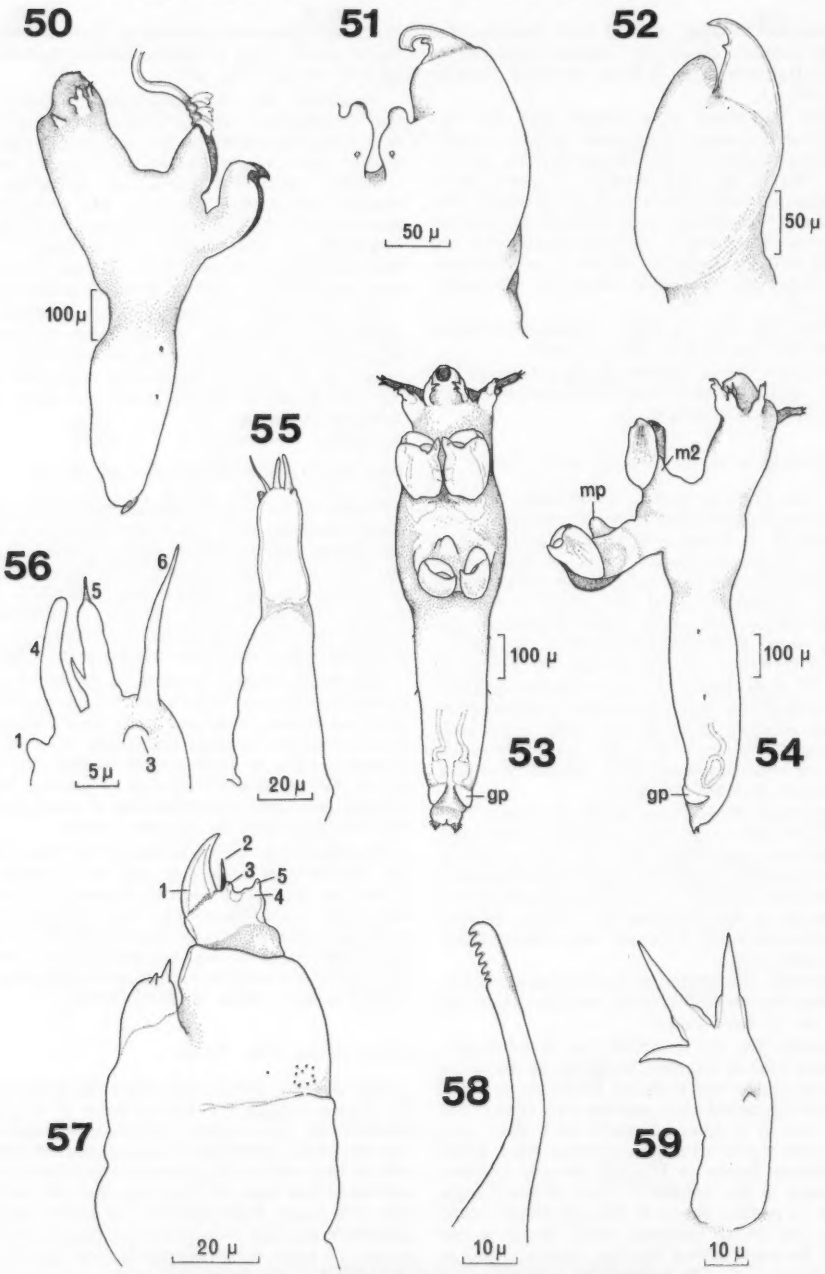
Unlike female, fourth chalimus male changed slightly during this stage (Fig. 50), resembling in most respects early fourth chalimus female, though with comparatively shorter trunk. Differences also present in development of bases of second maxillae and maxillipeds. Ventral surface of cephalothorax inflated, appendages now arising out of summit of distinct eminence. Average total length (in μ, based on 15 specimens) 972 (880–1020).

Appendages similar to those of female, except for second maxillae and maxillipeds. Second maxillae (Fig. 51) fused at bases, short and squat, medial margins with conical processes at about half length, processes of two appendages almost meeting in midventral line. Maxilla greatly narrowing distal to level of process. Terminal claw of appendage resembling that of preceding stages, but with short denticle on inner margin.

Maxilliped (Fig. 52) less advanced than that of female, less heavily sclerotized and with more slender base. Process in distomedial corner rounded and concave, with finely corrugated surface. Claw simpler than that of female appendage, with short triangular process at about half length of inner margin, long axis nearly in line with that of corpus, so that entire appendage often appears chelate rather than subchelate.

Adult Male (Fig. 53–64)

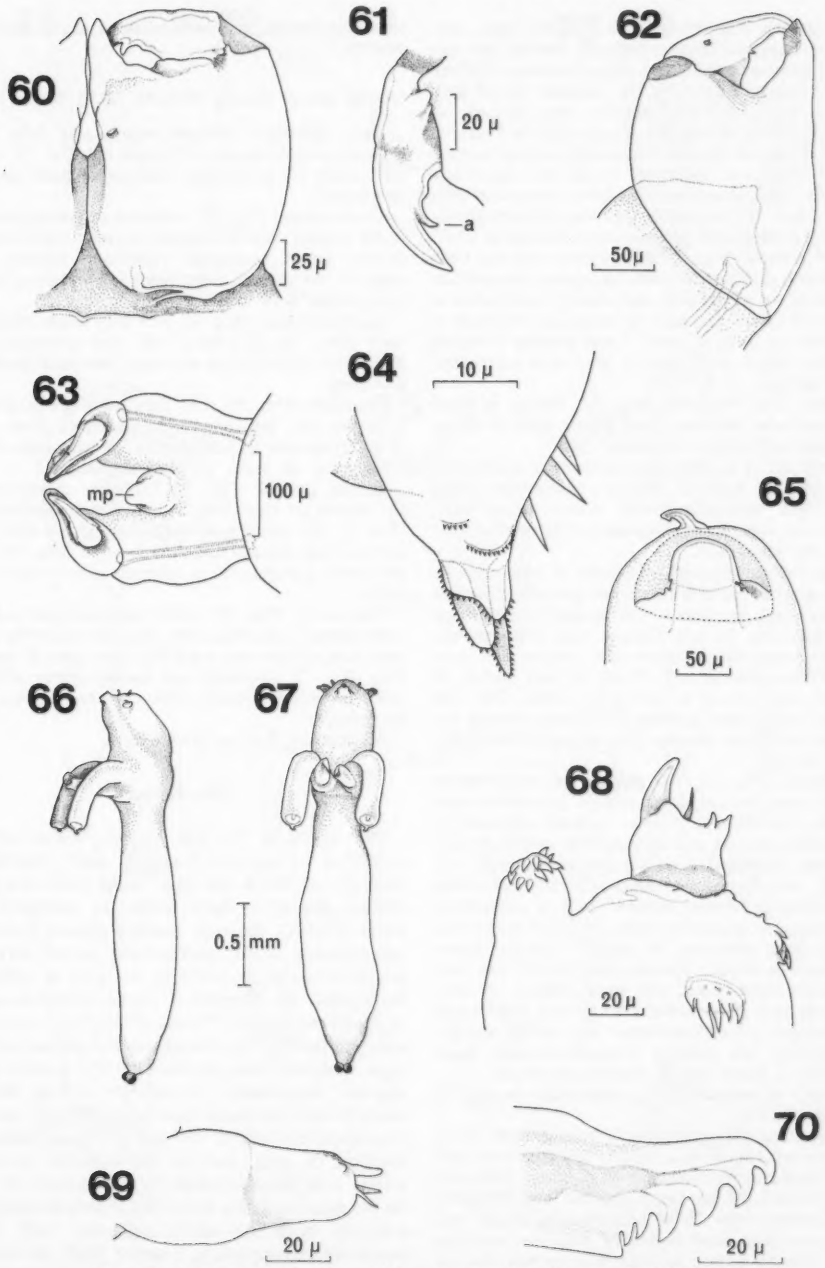
Body divisible into cephalothorax and trunk (Fig. 54) by shallow constriction behind bases of maxillipeds. Anterior to constriction, cephalothorax subdivided into two parts: narrow and cylindrical anterior associated with antenna and buccal apparatus, and inflated posterior associated with bases of second maxillae and maxillipeds (Fig. 53). Trunk about half of body length, cylindrical, posteriorly tapering. Near posterior extremity, on ventral surface of body, two crescentic sclerites, genital plates (Fig. 53, 54, gp), protecting orifices of genital ducts. Average length (in μ, based on 15 specimens) 1128 (1060–1240).



FIGURES 50–59. *S. californiensis*, fourth chalimus and adult male.

FIG. 50. Male, fourth chalimus, lateral; FIG. 51. Second maxilla, anterior; FIG. 52. Maxilliped, anterior; FIG. 53. Adult male, ventral. FIG. 54. Same, lateral; FIG. 55. First antenna, entire, medial; FIG. 56. Same, tip, dorsal; FIG. 57. Second antenna, lateral; FIG. 58. Mandible, lateral; FIG. 59. First maxilla, lateral.

[gp, genital plates; m2, second maxilla; mp, mediative process; other explanations in text]



FIGURES 60–70. *S. californiensis*, adult male, fourth chalimus and adult female.
FIG. 60. Male, second maxilla, posterior; FIG. 61. Same, claw, anterior; FIG. 62. Maxilliped, lateral; FIG. 63. Both maxillipeds, anterior; FIG. 64. Caudal ramus, ventral; FIG. 65. Fourth chalimus female, tip of second maxilla, lateral; FIG. 66. Fourth chalimus female without maxillary bridge, lateral; FIG. 67. Same, ventral; FIG. 68. Adult female, second antenna, rami, lateral; FIG. 69. First antenna, medial; FIG. 70. Mandible, tip, lateral.
[a, secondary tooth; mp, mediative process]

First antenna (Fig. 55, 56) with rounded apex, digitiform, in some specimens indistinctly divided into two parts by transverse constriction. Apical armature of three setae and two tubercles (Fig. 56). Medial side of apex with stout, blunt seta 4 and tubercle 1 near its base; on opposite side long, slender seta 6 with tubercle 3 near its base. Single seta of complex 5 in apical position, varying in shape from one specimen to another, secondary outgrowths and attenuated tips being present in only some of them. (It is possible that they present abnormalities of development or result from damage in vivo.)

Second antenna (Fig. 57) only slightly differing from that of fourth chalimus. In some specimens denticulation absent from tip of exopod and changes noticeable in armature of distal segment of endopod. Tubercle 3 now present at base of hook 1 and process 5 arising from more ample and rounded process 4 on ventral side of segment.

Mandible (Fig. 58) fairly long, flat, flexing in basal half, armed with six, less often seven, teeth of about equal shape and more or less equal size.

First maxilla (Fig. 59) characterized by changes in relative sizes of terminal papillae, ventralmost being smallest and well-differentiated apical setae being present on all three. Exopod represented by small conical swelling on lateral wall.

Second maxilla (Fig. 60, 61) fused at base with its opposite number but able to execute laterally divergent movements, both members of pair arising from common inflated base (Fig. 54, m2). Corpus oval, with pointed, triangular outgrowth in distomedial corner and duct orifice at base of outgrowth. Cavity in inner surface of outgrowth receives tip of subchela. Latter (Fig. 61) with very broad base, tapering to slightly curving tip. Secondary dentiform process (Fig. 61, a) on its inner, concave margin.

Maxillipeds (Fig. 62, 63) arising out of common base, with short, soft, conical mediative process between them (Fig. 54, 63, mp). Corpus inclined obliquely to base, turning inwards and approaching medial margin of opposite maxilliped (Fig. 63). Corpus roughly oval (Fig. 62), with prominent outgrowth of distomedial corner extending beyond anterior level of appendage. Surface ridged in spoon-like cavity of tip of outgrowth, providing good purchase for tip of subchela. Latter resembling true chelate stucture, with broad base and sharp flexure close to tip, with small process on inner margin and very short basal seta. Corpus linked with ventral surface of cephalothorax by strong, straight apodeme (Fig. 63) running through common base, very visible in living and in cleared specimens.

Thoracic legs represented by single setae on wall of trunk (Fig. 53, 54).

Caudal ramus (Fig. 64) conical, bearing three short, naked setae on lateral wall, distally splitting into three laminae fringed by continuous rows of sharp, triangular denticles, dorsal lamina being shortest and broadest, two succeeding ones being progressively longer and narrower, so that distal halves of both are ventrally exposed. Ventral surface of cone bearing two rows of denticles, similar to those fringing laminae. Size and shape of laminae differ somewhat from specimen to specimen. In many, laminae apparently subjected to wear and damage, with parts missing and gaps in marginal armature.

YOUNG ADULT FEMALE (FIG. 68–72, 74–80)

Only difference between young and fully mature females is development of trunk (cf. Fig. 74 and 80) and mode of attachment. All appendages now fully developed.

First antenna (Fig. 69) retarded in growth, relatively much smaller than in younger stages. Apical armature seldom intact, sometimes completely missing, shape squatter and broader. Subdivision into basal and terminal parts either very indistinct or absent.

Second antenna (Fig. 68) with fully adult armature of both rami. Tip of exopod with very prominent, long, and robust apical spines obscuring two usual papilliform processes.

Mandible (Fig. 70) with fully adult width (compare with Fig. 45), but otherwise unchanged. First maxilla (Fig. 71) broader at base than at tip, with well differentiated setae on three terminal papillae.

Second maxilla (Fig. 74, 75) more elongated, with tip devoid of claw and with exposed maxillary plug (Fig. 75, pl) partly protruding through slit-like orifice. In specimens already attached to bulla (Fig. 79), length of second maxillae, when relaxed, might reach that of trunk.

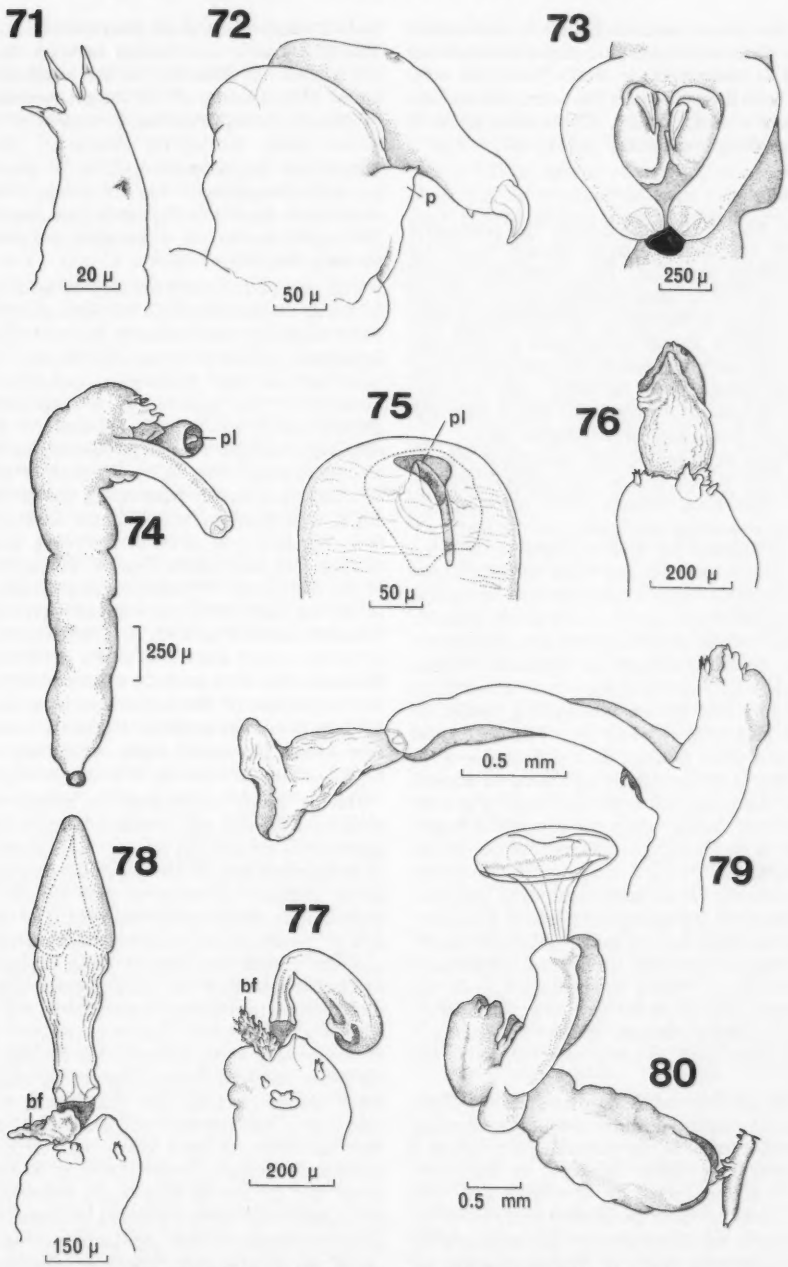
Maxilliped (Fig. 72) with well-developed subchela, with distinctly delimited short claw. Small papilla present near base of claw and basal seta near base of subchela. Palp (Fig. 72, p) conical and usually tipped with three small processes (though often only one or two might be present).

No thoracic legs or caudal rami.

Life History

Our study of the life history began with the collection of egg-sacs from the adult females and attempts to hatch the eggs under laboratory conditions. Placed in Petri dishes, in unfiltered fresh water at 10 C, the eggs hatched almost invariably, the duration of the prehatching period depending on the stage of maturity at the time of collection. In general, the progress of maturation was similar to that described by Friend (1941) for *S. salmoneus* and was marked by changes in the pigmentation of eggs. Hatching was preceded by the rupture of the egg-sac. Movements of embryos within the eggs could be seen for some time before the sac ruptured. A longitudinal split in the wall of the sac, caused by swelling of eggs due to the osmotic uptake of water, was accompanied by dehiscence of eggs. As the eggs burst, the somewhat crumpled copepodid emerged from the partly collapsed shell, simultaneously accomplishing a moult from the nauplius into the copepodid stage and hatching from the egg.

The course of the life history will be described below with division into stages, as in the preceding



FIGURES 71–80. *S. californiensis*, fourth chalimus and adult female.
FIG. 71. Adult female, first maxilla, lateral; FIG. 72. Maxilliped, anterior; FIG. 73. Fourth chalimus, maxillary bridge, ventral; FIG. 74. Young adult female, lateral; FIG. 75. Second maxilla, tip; FIG. 76. Bulla at early stage of eversion; FIG. 77. Same, at later stage; FIG. 78. Bulla fully everted; FIG. 79. Young female newly attached to bulla, anterior half, lateral; FIG. 80. Fully developed female, with male attached.
[bf, escaping body fluids; p, palp; pl, maxillary plug]

section. The events described and, in particular, the timing of the events are true of our observations conducted at between 11 and 12 C. Higher temperatures have little effect on the events themselves, but accelerate their timing. The entire cycle is represented diagrammatically in Fig. 88.

COPEPODID

Without finding a suitable host, the copepodid can survive for about 2 days, though many die before reaching that age. The copepodid spends the first 30 min or so after hatching almost motionless on the bottom of the Petri dish. Small movements of the appendages appear to be aimed at grooming and fully extending the cuticle. At the end of that period copepodids begin to swim very actively, propelling themselves through the water with the help of their two pairs of swimming legs.

The resting position of the swimming legs is shown in Fig. 1 and 2. The legs point obliquely forwards, their long pinnate setae reaching the level of the transverse subdivision of the forebody (Fig. 1). (It should be noted, however, that the legs reverse their position and begin to point backwards immediately after death, due to contraction of the powerful flexor muscles. It is in this position that they are usually depicted.) Forward progression is effected by the simultaneous backward strokes of both pairs of swimming legs, their movements being synchronized by the interlocking system of interpodal bars (Fig. 15 and 16). The copepodid proceeds in a jerky fashion, each jerk caused by a single stroke of its swimming legs. One or several strokes in rapid succession are followed by a brief interval of rest, during which the copepodid begins to lose speed and sinks towards the bottom of the vessel. Sinking along an arc-like path, the copepodid meets the substrate at an angle that places the three pairs of prehensile appendages (the second antennae, the second maxillae, and the maxillipeds) into simultaneous contact with it. The bases of the appendages are set in a plane oblique to the long axis of the cephalothorax (Fig. 1). At the same time, the anterior margin of the cephalothorax, with its small sensory setae, also finds itself in close proximity with the substrate.

Such contact with be bottom commonly elicits a rapid and immediate upward movement, carrying the copepodid towards the surface, again along a curving path. The change of depth in the latter instance is accomplished by dorsiflexion of the hindbody. This change in the relative position of the two tagmata is made possible by the structure of the intervening joint (Fig. 4). The contraction of the dorsal longitudinal muscles causes the two heavily sclerotized, lateral processes of the hindbody to slide over similar processes of the forebody. The thin cuticle in a lacuna between them yields and allows the deflection of the hindbody to take place. The structure of this hinge does not appear to permit any appreciable movement in the horizontal plane. Horizontal change of direction is effected by the copepodid tilting to one side and changing the plane of the arc along which it will move when flexing its hindbody in dorsal direction. We were unable to determine the mechanism causing this lateral tilt.

The copepodids were induced to settle on pieces of fins or on the skin of immobilized fry of *O. nerka*. Their settling on the surface of the host's tissues was sometimes preceded by several swoops, brief contacts with the host, followed by immediate upward movement. The attachment process differed in details, but it was usually initiated by a forceful downward stroke of the prehensile hook of the second antenna (Fig. 6), reminiscent of the action of a pick, and clearly penetrating the surface of the tissue. The powerful claws of the second maxillae (Fig. 11) were next to be driven home in a similar manner. The maxillipeds (Fig. 14), the most posterior of the three pairs of prehensile appendages, seemed to be the least active, sometimes participating in the attachment by hooking in at points posterolateral to the tips of the second maxillae. More commonly, however, they were held, claws extended, under the ventral surface of the body. Touching the skin of the host at a point posterior to the parasite's mouth cone, they performed light, scratching motions, acting usually alternately, less commonly together.

Having firmly established its contact with the surface of the fish, the copepodid enters the second phase of its attachment process. This phase consists of movements over the surface of the fish, apparently in an attempt to locate a spot suitable for the implantation of the frontal filament. The copepod is able to move both forwards and backwards, in either case the change of position being carried out by successive detachment of the prehensile appendages, their forward (or backward) movement, and reattachment in a new position. The most common sequence of movements is as follows: one second antenna, the other second antenna, one second maxilla, the other second maxilla. The maxillipeds sometimes take part in this sequence and are the last to change their position. At other times they do not act as attachment organs, continuing their scratching activity near the mouth cone of the parasite. At each new locality the surface is tested by contact with the anterior margin of the cephalothorax and by the tip of the mouth cone. The first antennae do not appear to play any part in this process. The two pairs of swimming legs also remain immobile, maintaining

their resting positions. In so doing they sometimes obstruct the forward movement of the copepodid, particularly when it crosses the fin. The tips of the pinnate setae engage each fin ray and have to be jerked upwards by a slight movement of the entire body.

The factors influencing the choice of site by the copepodid are impossible to determine at present. (They involve the presence of a solid subdermal support, such as a fin ray, a rod of a gill filament, a scale, or a bone, to which the terminal plug of the frontal filament is cemented.) Having found a suitable spot, the copepodid straddles it by firmly planting the claws of the second maxillae and the hooks of the second antennae. The scratching acitvity of the long and slender claws of the maxillipeds intensifies and results in the excavation of a sizeable cavity in the tissue of the host, between the points of penetration of the second maxillae. The copepodid might interrupt this activity at any stage before its completion and resume its searching behavior. Some of the experimental copepodids started as many as three separate excavations. It appears, however, that this process exhausts the parasite, an excavation taking up to 90 min to complete. Towards the end of this activity, it is often interrupted by brief periods of rest and almost complete immobility.

When the cavity reaches an appropriate size, it is tested again by the tip of the mouth cone and by the anterior margin of the cephalothorax. The latter is applied to the cavity, vigorously pushed into it, and remains there, while the two pairs of prehensile appendages (the second antennae and the second maxillae) flex repeatedly, performing movements that are best described as "push-ups". This process is accompanied by rapid detachment of the large terminal plug of the frontal filament (Fig. 3) from the surrounding cuticle. The plug is firmly inserted into the cavity in the host tissue and is cemented to the supporting skeletal structure. Cement substance is provided by the frontal gland and appears to harden very rapidly. Its nature is as yet unknown. Taking usually about a minute, the entire process involves some loss of fluid (cement substance?) from the hole in the anterior margin. The extent of loss varies from one copepodid to another and appears to be quite severe in some. The copepod now begins to walk backwards in the manner described above. As it does so, it pulls out the thin end of the frontal filament (Fig. 3) and uncoils it, until the entire filament is evaginated and the small basal plug changes its position by 180°. In many copepodids the process of development does not progress any further. Whether this is due to the now permanent attachment to a site that proves unfavorable on closer contact, or the damage to the anterior margin of the body associated with the detachment of the frontal filament, is difficult to say. At any rate, the very first stage of attachment by the frontal filament seems to be a point of danger in the life history of the parasite.

In a 500-ml beaker of water with one 5-cm long *O. nerka*, all copepodids were attached and had their frontal filaments evaginated within 3.5 hr. Under normal conditions, when the temperature and the water flow are close to those occurring in nature, the evagination of the filament is followed almost immediately by the beginning of the moult. The moult itself is normally accomplished within about 30 min. The duration of the attached copepodid stage, however, depends largely on the length of time spent in searching for a suitable spot for attachment, which can be as long as 10 hr or, possibly, 24 hr. Under the microscope, in stagnant water, the moult itself can take a very long time (in one instance moulting was not concluded for $2\frac{1}{2}$ days).

Safely anchored to the host by its frontal filament, the copepodid begins to moult by withdrawing its prehensile appendages (first the maxillipeds, then the second maxillae) from the copepodid cuticle. The maxillipeds assist the withdrawal of the second maxillae by pushing from within against the cuticle at their bases. The hindbody then withdraws from the narrow sheath of the copepodid cuticle. Folded upon itself, it rests on the transverse shelf of cuticle, where it expands into the wide part of the hindbody. The cuticle is next detached from the margins of the base of the frontal filament. Working their way forward within the copepodid cuticle, the second maxillae move toward the base of the filament. Equipped now with short, barbed claws, they penetrate the basal plug and become attached to it with the assistance of some more secretion of the frontal gland (Fig. 18, ad). A firm fusion between the plug and the claws is followed by the detachment of the frontal filament from the anterior margin of the body. The action is aided by the movements of the second antennae, which support the base of the filament, having previously retracted from the copepodid cuticle. The first chalimus must emerge from the copepodid cuticle by sliding out, head first, through its torn anterior margin. This is accomplished by a succession of contractions and expansions of the cephalothoracic region, accompanied by pushing of the posterior part of the body, initially folded and gradually assuming its straight shape. The contractions loosen the cuticle around the anterior part of the body, whereas the flexions of the posterior end propel it outwards, out of the sheath of the old cuticle. Under natural conditions

this is aided by the current of water pushing the cuticle backwards.

First Chalimus

The duration of this stage is about 12 hr. Its most important achievement is the transfer of the attachment by the frontal filament from the anterior margin of the body to the tips of the second maxillae. Although in some specimens it takes place within the cuticle of the copepodid stage, others do not accomplish it until that cuticle has been shed. Consequently, one finds chalimus I parasites still attached by their anterior margins. When transferring the base of the frontal filament outside the old cuticle, the progress of the change is similar to that described. The short second maxillae (Fig. 17) have to negotiate the prominent mouth cone on their way back with the filament, an action accompanied by a good deal of stretching and pulling, as well as by bending the cone in a posterior direction. Within about 36 hr from the initial contact with the host, the parasite is in the process of moulting into the second chalimus stage (Fig. 88).

Second Chalimus

The process of moulting into the second chalimus stage is, in general, similar to that described for the previous moult. The most important feature of the process is the reattachment of the parasite to the frontal filament. Prior to withdrawing the second maxillae of the new stage from the cuticle of chalimus I, they are rather laboriously transferred back to the frontal region of the body over the prominent obstacle of the buccal cone. By this time some degree of cuticular disintegration has taken place and some of the buccal appendages might be free, particularly the second antennae, which are very active in the process of transfer. As the second maxillae maneuver the proximal end of the frontal filament into the area where the frontal gland debauches, a drop of viscous secretion exudes from the orifice; the end of the filament becomes attached to that drop. The second maxillae contract and press the filament firmly into the secretion, vigorously aided by the second antennae. Another result of maxillary contraction is the withdrawal of these appendages from the cuticle of the preceding stage. Still inside the old exuvium, the second maxillae work their way around the mouth cone until they reach the frontal region, where the new barbed claws are inserted into the still tractable drop of secretion. The drop soon solidifies and is gently detached by the combined action of the second maxillae and second antennae. Its proximal end retains an impression of the convex wall of the frontal region (Fig. 27). The second maxillae then move backwards over the buccal cone to their normal position.

In this and subsequent chalimus stages the process of reattachment is completed while the parasite is still contained within the old cuticle. The latter continues to swing from the filament by the empty sleeves of the old second maxillae, forming a bag with the moulting chalimus in it. Until the fresh secretion hardens and the second maxillae return to their normal position, this bag cannot be removed from the posterior part of the body. The removal of the exuvium is a laborious process. On its completion, the tattered exuvium usually remains suspended from the base of the frontal filament. The first task of each newly-moulted chalimus is to cut and tear away the old exuvium by employing its mandibles. This process often begins before the cuticle is fully shed and is aided by water currents. Normally, only the old pairs of second maxillary claws remain, embedded in successive drops of secretion at the bottom of the frontal filament.

The duration of the chalimus II stage is about 12 hr so that within about 48 hr from the initial contact with the fish the parasite might begin to moult into the following stage.

Third Chalimus

The activity of the parasite during this stage is similar to that occurring in the previous stage, highlighted by the transfer of the second maxillae to the new drop of secretion (Fig. 34), performed as previously. At this stage the first differences can be observed between the sexes. Whereas the duration of the third chalimus male is in the order of 24 hr, that of the female is about twice as long. Careful observation also allows one to distinguish between sexes morphologically, at least by the end of the stage. The posterior extremity of the late chalimus III male is distinguishable by a slight dorsiflexion.

Fourth Chalimus Male

The development of the male during this stage largely resembles that of the previous chalimus stages. The one essential difference between it and those stages is the fact that after about 40 hr the male moults, without repeating the process of reattachment, into a young adult. The process of this metamorphosis is related in the section on the adult male. Figure 43 shows the proximal end of a frontal filament to which a fourth chalimus male is attached. Note empty cuticle of claws of three earlier stages.

Fourth Chalimus Female

Having reattached to the bottom of the frontal filament, by now marked with three pairs of claws embedded in successive drops of secretion (Fig. 43), the female embarks on a crucial part of its metamorphosis into an adult specimen. The process begins with a unique activity, eventually resulting in the reversal of positions of the second maxillae and the maxillipeds. The maxillipeds, eminently functional at this stage (Fig. 46), grasp the surface of the host with their hooked subchelae. One at a time, they then "step over" the linked tips of the second maxillae so that their position comes to resemble that shown in Fig. 42. Bringing into action its second antennae, the female begins to move forwards, alternating its attachment in a manner similar to that described for the copepodid stage, dragging behind it the frontal filament. This forward progress is not continuous. Periods of rest intervene. The female continues to grow, its trunk gradually elongating and eventually imparting to it the shape shown in Fig. 66. The second maxillae also elongate vigorously, their length in relation to that of the body becoming greater, their bases gradually slipping backward in relation to those of the maxillipeds.

This continuous movement brings about stretching of the frontal filament, which becomes taut and prevents further progress. Firmly hooked with the second antennae and maxillipeds, the female strains at the filament, contracting its second maxillae and tugging repeatedly at its base. Finally, the base of the filament breaks, in most instances along the line of junction of the last drop of secretion with that of the preceding moult, leaving it still linking the tips of the second maxillae (Fig. 73) as the structure we have referred to as the maxillary bridge. The entire operation is fraught with difficulties; in particular, by no means always does the frontal filament break in this way. In Fig. 66 and 67 we see females that, by tugging at the filament, succeeded in slipping the tips of their second maxillae out of the base of the filament. These appendages are now free and wave about in apparently aimless fashion. It is possible that this outcome of the process of separation from the filament is influenced by the size of the claw. A normally embedded claw is shown in Fig. 48. It is comparatively larger than that in Fig. 65, taken from a female without a maxillary bridge. Our observations have shown that between 10 and 15% of females do not retain a maxillary bridge in the process of releasing the frontal filament.

The duration of the chalimus IV stage of the female is very long, extending to as long as 2 weeks from the moment of contact with the host. Its length appears to depend on the time taken by chalimus IV to locate a suitable site for its final attachment. When such a site is located, the parasite begins to excavate a cavity in which the bulla of the adult will eventually be implanted. This task takes a long time as the parasite can no longer use its second maxillae (partially immobilized by the maxillary bridge) or its maxillipeds (now the main prehensile appendages). The excavation is accomplished with the aid of the buccal appendages and, to a lesser extent, the second antennae. On some occasions excavation might be interrupted and another place sought out before the female is finally committed to its definitive site of attachment.

Adult Male

The emergence of the adult male from the cuticle of its chalimus IV stage takes place in a manner similar to that occurring in the preceding moult. The only difference is in the fact that the second maxillae of the adult do not embed in the base of the frontal filament, though they grip it with their subchelae. Some adult males are found grasping the exuvia of chalimus IV, still swinging from the end of the filament. At the nearest opportunity, when the agile second antennae come into contact with the surface of the host, the male begins to relinquish its hold on the filament, transferring it to the tissues of the host, engaging in turn by the second maxillae and the maxillipeds.

Once firmly in contact with the surface of the host, the male begins to wonder in quest of the female. Its mode of progression is best understood by referring to Fig. 81–84. The posterior part of the ventral surface of the cephalothorax is inflated to form a common base for the second maxillae and the maxillipeds, splayed out from its corners to produce a four-point attachment. The movement begins with the forward stretching of the anterior part of the cephalothorax to the limit of its extensibility (Fig. 81). The hooks of the second antennae (Fig. 57) are implanted in the host tissues. The second phase of the movement comes with the upward arching of the trunk, bringing to mind the looping movement of an inchworm (Fig. 82). The posterior extremity of the trunk is thereby brought close behind the bases of the maxillipeds. The caudal furca is then rammed into the surface of the fish. The structure of the caudal rami (Fig. 64) is such that all three of its terminal laminae are simultaneously placed in contact with the substrate and, aided by two rows of denticles in front of them, prevent the posterior end of the male from slipping backwards during the third phase of the movement. This

phase (Fig. 83) comprises simultaneous contraction of the longitudinal muscles of the cephalothorax, disengagement of the second maxillae and maxillipeds, and a downward-pressing movement of the trunk. The net effect is a forward shift of the maxilla-maxilliped platform. It continues to move until the anterior margins of the second maxillae find themselves close behind the buccal region. Both pairs of prehensile appendages hook again into the surface of the host. The next cycle (Fig. 84) begins with another forward-stretching movement of the cephalothorax, preceded by a moment of rest shown in Fig. 84.

On encountering a female, the male becomes attached to its genital region and begins fertilization, to be described in a separate section below.

Adult Female

The moult that transforms a female chalimus IV into a young adult takes place in conditions completely different from those that prevailed in earlier moults. The parasite is no longer attached by its frontal filament. It grips the host with its maxillipeds aided by the second antennae. The second maxillae, now elongated, cylindrical appendages, are confined underneath the body by the maxillary bridge. The process of moulting begins with the usual rupturing of the cuticle in the frontal area and liberation of the second antennae, which for a time act as the most important attachment organs, while the maxillipeds slip out of the old cuticle. The biggest problem is long second maxillae, no longer able to pull against the frontal filament in their efforts to shed the chalimus cuticle. One could compare it to an attempt at pulling one's arm out of a sleeve without any restraint being placed on the cuff. In this instance restraint is provided by the maxillary bridge (Fig. 73), allowing one second maxilla to be withdrawn and the exuvium to slide down far enough to free the other one. (Assistance of water currents is of some importance here.) The significance of the maxillary bridge is clearly shown by the fact that all those females that failed to retain it on relinquishing their frontal filaments are incapable of moulting again and usually disappear from the surface of the host within 24 hr after their metamorphosis into chalimus IV. This obstacle in the path of development reduces the adult parasite population by 10–15%.

During the process of moulting into adult, or immediately after its completion, the parasite begins to evert its bulla, the definitive attachment organ. Having developed during the course of the chalimus IV stage, the bulla is located in the space previously occupied by the frontal filament. It is folded upon itself, crumpled and much smaller than upon emergence.

The female is now at a very critical stage of development. The eversion of the bulla is accompanied by damage to the frontal region and, almost invariably, some loss of body fluids (Fig. 77, 78, bf). It is dangerous and can be fatal. In the course of experiments we found a number of dead females with bullae at different stages of eversion. In one of them rupture occurred on the side of cephalothorax, near the base of the second antenna.

The "normal" eversion of the bulla can have one of two courses. The unfolding and eversion can occur simultaneously (one such partly everted bulla is shown in Fig. 76). On the other hand, the bulla can be completely everted, while still in folded condition (Fig. 77), to straighten subsequently (Fig. 78). A completely everted bulla (Fig. 78) is a compressed, clavate structure, with numerous longitudinal folds and tip only slightly wider than base. The division between the future manubrium and anchor (Kabata and Cousens 1972) is distinguishable, but the latter is still closely compressed. The bulla is attached to the frontal region by strands of loose tissue.

We were unable to find whether the distal end of the bulla possesses any adhesive properties at this stage. At any rate, placed in the cavity of implantation by the movement of the cephalothorax, it remains in it, pressed firmly by similar movements. When the cephalothorax draws away, the strands of tissue connecting it to the bulla are broken and the bulla remains implanted, the base of the manubrium facing the parasite. The female, still attached to the host only by its maxillipeds, begins broad, sweeping movements with its second maxillae in the area of implantation. Coming in contact with the bulla, the maxilla is lined up with the appropriate duct opening in the base of the manubrium. The maxillary plug (Fig. 75, pl) is firmly inserted into the opening. This contact becomes permanent due to the action of a cement substance produced by the maxillary gland. Solidified at the border between second maxilla and the bulla, it fuses them for the duration of the parasite's life. The attachment of the other second maxilla follows and the definitive attachment is nearly completed.

It appears that this process is not accomplished without some risks and difficulties. It must be completed within a relatively short time, often accompanied by the other activities associated with the moulting process. The manubrium must be found, its duct orifices must be clear of extraneous matter, and the contact between the bulla and the second maxilla must be maintained long enough to permit the secretion of the maxillary gland to be

produced, delivered to the tip of the appendage, and solidified. One finds implanted bullae, obviously abandoned by females that failed to attach and complete their life cycles. On occasions, mature females can be found attached to the bulla by one maxilla only, the other still performing apparently purposeless movements.

The condition of the freshly implanted bulla differs little from that at the time of eversion. As soon as the second maxillae establish their permanent contact with the manubrium, the bulla begins to expand within the tissues of the host. The expansion is due to the transport of substances secreted by the maxillary glands and entering the bulla through the openings in the maxillary plugs. In Fig. 79 the bulla is seen partly inflated, that in Fig. 80 has already attained its definitive size and shape.

That the maxillary secretions are, in fact, responsible for the inflation of the bulla is clear when one observes specimens that are connected with the bulla by one maxilla only. The maxilla that failed to make the connection becomes discolored and distended with accumulated secretion, appearing as a plug of dark yellow or pale brown color visible through the cuticle. In such females, the half of the bulla corresponding to the unattached maxilla becomes only partially inflated (via the duct connected with the attached maxilla) and is clearly distinguishable from the fully inflated connected half. (We did not determine whether young females with only one maxilla attached can survive to egg-bearing stage, but none of the specimens in that condition carried egg-sacs.)

Having successfully completed its definitive attachment, the female continues to grow, its trunk developing in length and, especially, in width. Many females at this stage have males fixed in the genital area.

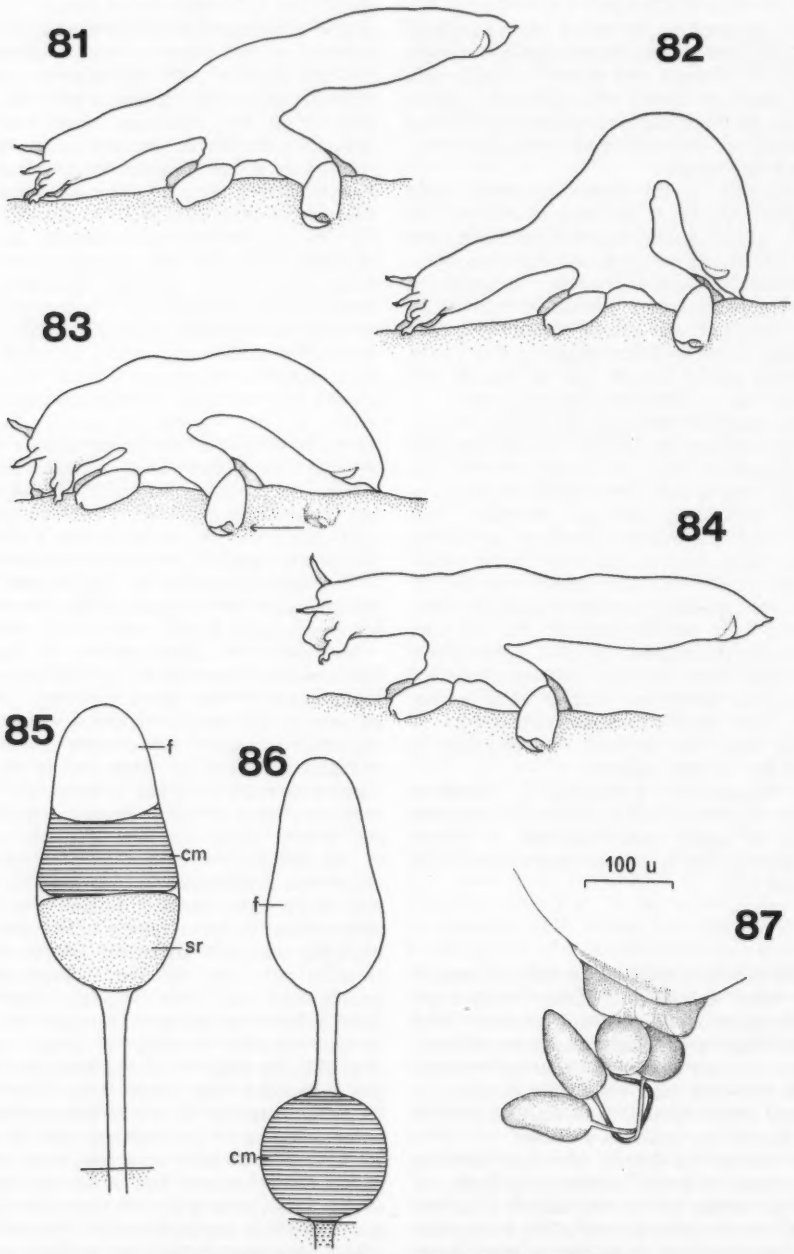
Mating

A female can be fertilized long before it attains maturity. Many females at chalimus IV stage are found with spermatophores already attached. This is especially true for the high density populations, where males are fairly abundant. Under experimental conditions, involving high population densities on experimental fish, at least 75% of the females were fertilized during the chalimus IV stage.

Having located the female, the male attaches itself to, or near, its genital process by gripping the cuticle of the female with its maxillipeds (Fig. 80). Only the tips of the subchelae and of the distomedial processes are involved; therefore, a fairly large space remains enclosed by the maxillipeds (Fig. 63). The process of fertilization begins with the trunk of the male arching forwards in a manner similar to that occurring during locomotion. The posterior extremity of the trunk is brought close to, and past the level of, the maxillipeds, through the enclosed space. The genital plates of the male (Fig. 53 gp) find themselves opposite the vaginal orifices of the female and are maintained in that position by friction between the female cuticle and the denticulated plates of the male caudal laminae. The spermatophores, when extruded, appear as in Fig. 85. A pyriform bulb, broader distally and extending into a thin tube, contains a sperm packet (sr) at its distal end, a quantity of cement substance (cm) in the middle part, and transparent fluid (f) in the narrower proximal part. On extrusion, the thin terminal tube enters the vaginal orifice directly. On some occasions the mediative process (Fig. 63, mp) appears to act as a ram, assisting penetration of vagina by the tube. On other occasions it prevents an accidental slipping of the spermatophore below the level of the maxilliped through the space now vacated by the posterior end of the trunk. The buccal tube and the antennae make quick touching movements, apparently irrelevant to the process of fertilization. We have not been able to observe any manipulation of the spermatophores by the second maxillae, though Fasten (1914) reported that they were used for this purpose by the male of *S. edwardsii*.

Soon after the spermatophore is inserted, the sperm packet moves down the tube and enters the genital duct of the female, followed for a brief distance by the cement substance. This movement may occur as a result of an increase in volume of the transparent fluid in the distal part of the spermatophore by osmotic uptake of water, the increased pressure forcing the more proximal sperm packet and cement down the tube and into the ducts of the female. The cement seals off the vaginal orifice and accumulates outside it in the adjacent part of the tube, which it distends into a regular sphere linked by the remainder of the tube with the pyriform sac, now somewhat smaller (Fig. 86). It is in this form that the spermatophores are usually seen and were originally described. In recently fertilized females both vaginae are plugged by the spherical droplets of cement substance (Fig. 87), with tubes of varying length and pyriform sacs suspended from them. Soon, however, only the spheres remain, the rest of the spermatophores either dropping off or being removed by the male. We have seen, on some occasions, males vigorously pulling at the terminal sacs of the spermatophores already spent, holding them in their second maxillae and flexing their cephalothoraces in dorsal direction.

It is clear that the vaginal openings are sealed for life and that no further fertilization is possible. Fasten (1914) suggested that the female can be fertilized repeatedly, basing his opinion on the



FIGURES 81–87. *S. californiensis*, adult male and female.
FIG. 81–84. Successive stages in locomotion of male (diagrammatic); FIG. 85. Spermatophore, before fertilization (diagrammatic); FIG. 86. Same, after fertilization (diagrammatic); FIG. 87. Female genital region with discharged spermatophores.
[cm, cement substance; f, transparent fluid; sr, sperm packet]

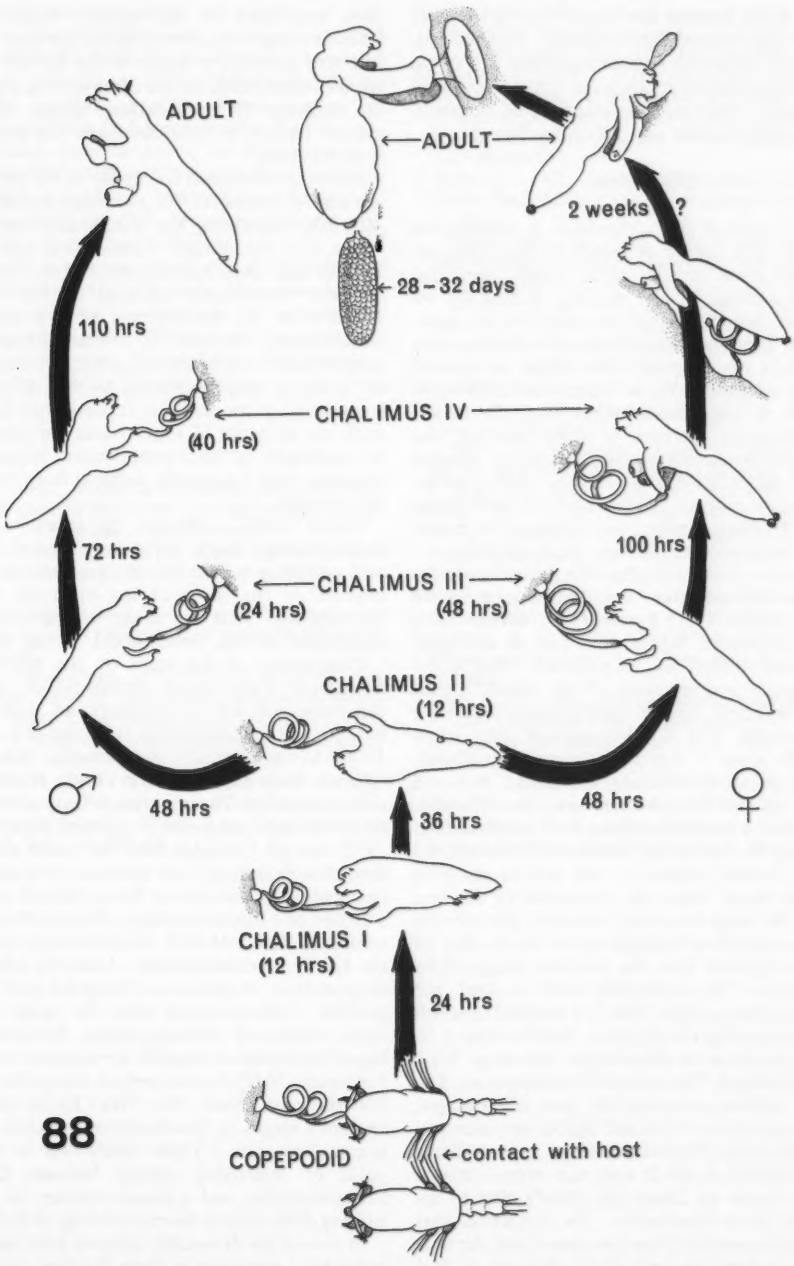


FIG. 88. *S. californiensis*, life cycle diagram (times in brackets denote duration of stages, those without brackets denote time from first contact with fish).

fact that some females are found with more than one pair of spermatophores attached. This, indeed, can happen. Sometimes spermatophores are being attached to the female at places far removed from its genital region. Only two spermatophores, however, can penetrate vaginae and effect fertilization.

Discussion

The life cycle of *S. californiensis* is summarized in Fig. 88 with timing of events as they occur at 11 C. The cycle consists of six stages, separated from one another by five moults. It must be remembered, however, that, in addition to morphological changes produced at each moult, other changes take place during some stages as a result of differential growth. These changes are particularly noticeable in long-lasting stages (e.g. the female fourth chalimus). Because of their presence, the ontogenetic development becomes more gradual and less a series of abrupt changes. Unfortunately, it is also made more complicated for the investigator who tries to piece together the course of the events without being able to observe them directly.

All previous authors dealing with the lernaeopodid life cycles labored under this disadvantage. Some of them (Wilson 1911; Fasten 1913; Savage 1935) were able to record only a small part of the cycle. Others were misled by fragmentary observation into incorrect interpretation of the events. Zandt (1935), for example, divided the life cycle of *Achtheres pseudobasanistes* (= *S. coregonorum*) into seven stages. His stage 2 was a chalimus, immediately following the free-swimming copepodid and still attached by the frontal region to the filament. We have seen a similar condition in *S. californiensis*. It is normal for the frontal filament to be transferred from the frontal region to the second maxillae during the moult from the copepodid to the first chalimus. In some instances, however, the parasite appears incapable of detaching the basal plug of the frontal filament from the anterior margin of its cephalothorax. The copepodid cuticle is shed, but the chalimus that emerges from it is doomed to perish without completing its life cycle. Zandt's stage 2 is, therefore, based on an abnormality. His stage 3 is a normal chalimus I. The cycle of *S. coregonorum*, like that of *S. californiensis*, consists, then, of six stages. (Zandt's correlation of text and figures was incorrect in some instances. Illustrations of the male chalimus IV were labelled as adult and vice versa.) Several comments made by Zandt are clearly due to insufficiently close observation. He suggested that the male is incapable of free movement and depends on swinging from the end of the filament to find a female. He did not observe the formation of the bulla and tried to corroborate Wilson's (1911) statement that it is formed by the increase in thickness and decrease in length of the frontal filament. On the other hand, he did observe (Fig. 50 and 53) the existence of the maxillary bridge, though he did not realize its significance and did not mention it in his text.

Dedie (1940) found six stages in the life cycle of the male *S. mattheyi* (=*S. edwardsii*), his description generally resembling the events described in this paper. For the female, however, he postulated a seventh stage that, initially attached by the filament, becomes permanently attached by the bulla. He did not see the development of this organ. The female, which releases its frontal filament at the stage of fourth chalimus (i.e. at stage 5), was believed by Dedie to remain attached to that filament until it switched to the bulla. It is more than likely that both the existence of stage 7 and the continuation of attachment to the frontal filament beyond fourth chalimus were incorrectly deduced from incomplete information.

Friend (1941), although he failed to observe developmental stages following the first chalimus and preceding adult, was quite correct in his description of the parts of the cycle he observed. To him also goes the credit of the first correct description of the development of the bulla.

Comparison of literature on the life cycles of *Salmincola* and related lernaeopodids confronts the researcher with a confusion of stages, designations, and interpretations. It led one of us (Kabata 1964b) to an incorrect conclusion that life cycles of different types might occur in closely related genera and even species. The opposite, in fact, can be expected. It has been suggested in a recent paper (Kabata 1972) that all Caligidae have life cycles comprising three free-swimming, four chalimus, and three post-chalimus stages (chalimus being defined as a stage attached by a frontal filament). It is interesting to find a life cycle also with four chalimus stages in a different family, Lernaeopodidae. Available information suggests that, in addition to Caligidae and Lernaeopodidae, Lernaeoceridae have life cycles with the same number of chalimus stages. Sproston (1941) found four chalimus stages in *Lernaeocera branchialis*. Jungersen (1911) found them in *Sarcotretes scopeli*. (On the other hand, Ho (1966) found only three chalimus stages in *Cardiodectes* collected from its intermediate host.) These similarities in ontogeny point to underlying affinity between Caligidae, Lernaeoceridae, and Lernaeopodidae, in spite of striking differences in the morphology of their adults.

In view of the drastically different host-parasite relationships occurring in these families, one cannot expect complete uniformity of their ontogenetic

processes. In Lernaeopodidae the number of both prechalimus and postchalimus stages is reduced (also of chalimus stages in the *Clavella* branch of the family), reducing the length of the period during which the parasite is either free-swimming of insecurely attached. In Lernaeoceridae the life cycle becomes more complex due to the introduction or the intermediate host and the attendant necessity for another period of free-swimming existence. They also, however, have fewer prechalimus and postchalimus stages than Caligidae.

All these types, however, are conceivably derived from an original basic life cycle. The value of this knowledge to those interested in the systematics, phylogeny, and general biology of the parasitic Copepoda is obvious. Its usefulness for practical purposes has already been stated.

DEDIE, O. 1940. Etude de *Salmincola mattheyi* n. sp., copépode parasite de l'omble chevalier (*Salmo salvelinus* L.). Rev. Suisse Zool. 47: 1–63.

FASTEN, N. 1913. The brook trout disease in Wisconsin waters. Rep. Wis. Comm. Fish. 1911–1912: 11–21.

1914. Fertilization in the parasitic copepod, *Lernaeopoda edwardsii* Olsson. Biol. Bull. (Woods Hole) 27: 115–126.

1919. Morphology and attached stage of first copepodid larva of *Salmincola edwardsii* (Olsson) Wilson. Publ. Puget Sound Biol. Sta. 2: 153–181.

1921. Studies on parasitic copepods of the genus *Salmincola*. Amer. Natur. 55: 449–456.

FRIEND, G. F. 1941. The life-history and ecology of the salmon gill-maggot *Salmincola salmonea* (L.) (Copepod crustacean). Trans. Roy. Soc. Edinburgh 60 (pt. II, No. 15): 503–541.

HO, JU-SHEY. 1966. Larval stages of *Cardiodectes* sp. (Caligoida: Lernaeoceriformes) a copepod parasitic on fishes. Bull. Mar. Sci. 16: 159–199.

JUNGERSEN, H. F. E. 1911. On a new gymnoblastic hydroid (*Ichthyocodium sarcotretis*) epizoic on a new parasitic copepod (*Sarcotretes scopeli*) infesting *Scopelus glacialis*, Rhdt. Vidensk. Medd. Naturhist. Foren. Kjøbenhavn 64: 1–33.

KABATA, Z. 1963. *Clavella* (Copepoda) parasitic on British Gadidae: one species or several? Crustaceana 5: 64–74.

1964a. Revision of the genus *Charopinus* Krøyer, 1863 (Copepoda: Lernaeopodidae). Vidensk. Medd. Naturhist. Foren. Kjøbenhavn 127: 85–112.

1964b. On the adult and juvenile stages of *Vanbenedenia chimaerae* (Heegaard, 1962) (Copepoda, Lernaeopodidae) from Australian waters. Proc. Linnean Soc. N.S.W. 89: 254–267.

1969. Revision of the genus *Salmincola* Wilson, 1915 (Copepoda: Lernaeopodidae) J. Fish. Res. Board Can. 26: 2987–3041.

1972. Developmental stages of *Caligus clemensi* Parker and Margolis, 1964 (Copepoda: Caligidae). J. Fish. Res. Board Can. 29: 1571–1593.

KABATA, Z., AND B. COUSENS. 1972. The structure of the attachment organ of Lernaeopodidae (Crustacea: Copepoda). J. Fish. Res. Board Can. 29: 1015–1023.

SAVAGE, J. 1935. Copepod infection of speckled trout. Trans. Amer. Fish. Soc. 65: 334–339.

SPROSTON, N. G. 1941. The developmental stages of *Lernaeocera branchialis*. J. Mar. Biol. Ass. U.K. 25: 441–466.

WILSON, C. B. 1911. North American copepods, Part 9, the Lernaeopodidae. Development of *Achtheres ambloplitis* Kellicott. Proc. U.S. Nat. Mus. 39: 189–226.

ZANDT, F. 1935. *Achtheres pseudobasanistes* n.n., syn. *Basanistes coregoni* (Neresheimer). Die postembryonale Entwicklung und geographische Verbreitung eines Lernaeopodiden. Zool. Jahrb. Anat. 60: 289–344.

Effects of Temperature on Responses of the Gonads of Green Sunfish (*Lepomis cyanellus*) to Treatment with Carp Pituitaries and Testosterone Propionate

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KAYA, C. M. 1973. Effects of temperature on responses of the gonads of green sunfish (*Lepomis cyanellus*) to treatment with carp pituitaries and testosterone propionate. J. Fish. Res. Board Can. 30: 905–912.

Previous investigations have demonstrated that stimulation of gonadal recrudescence in the green sunfish (*Lepomis cyanellus*) depends on the concurrent presence of long photoperiods (15 hr) and elevated temperatures (>15 C). The present investigation indicates that recrudescence can be stimulated in seasonally regressed ovaries and testes by injections of a crude extract of fish pituitary glands, and in testes by testosterone propionate, but only under elevated temperature. The low temperatures that block gonadal responses to long photoperiods also effectively prevent gonadal responses to administered hormones. These observations indicate that the responsiveness of the gonads of this species to stimulating hormones is markedly modified by temperature; however, the results do not obviate the possibility that secretion of gonadotropins by the brain–pituitary system may also be affected.

KAYA, C. M. 1973. Effects of temperature on responses of the gonads of green sunfish (*Lepomis cyanellus*) to treatment with carp pituitaries and testosterone propionate. J. Fish. Res. Board Can. 30: 905–912.

Il a été démontré, à la suite de recherches antérieures, que la stimulation de la recrudescence des gonades chez le crapet vert (*Lepomis cyanellus*) dépend de la présence simultanée de longues photopériodes (15 h) et de hautes températures (> 15 C). Le présent travail démontre que des testicules et des ovaires en état de régression saisonnière peuvent être stimulés par injections d'un extrait brut de glande pituitaire de poisson, et que les testicules peuvent l'être par le propionate de testostérone, mais seulement quand la température est élevée. Les températures suffisamment basses pour bloquer la réponse des gonades aux longues photopériodes bloquent également leur réponse aux hormones. Ces observations indiquent que la sensibilité des gonades du crapet vert aux stimulations hormonales est grandement modifiée par la température; cependant, ces résultats n'excluent pas la possibilité que la secrétion de gonadotropines par le système cerveau–corps pituitaire soit également affectée.

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EXPERIMENTAL studies have indicated that photoperiod and temperature can have important roles in regulating the seasonal reproductive cycles of fishes, although the relative importance of these factors may differ among species (De Vlaming 1972a). Although the involvement of the brain–pituitary axis in responses of gonads of fishes to photoperiod seems well accepted (Atz 1957; Hoar 1969; Baggerman 1972), there is little evidence on physiological bases for effects of temperature. Temperature could influence production of gonadotropic hormones by the pituitary (as apparently does photoperiod), could modify the responsiveness of the gonads to gonadotropic hormones, or could possibly have a direct effect on the gonads, independent of involvement of pituitary hormones (Atz 1957; Lofts et al. 1968). Atz (1957), after summarizing the few relevant earlier studies, suggested that their results indicate that failure of gametes to mature at low temperatures results from a deficiency of endogenous gonadotropin. However, low temperature has also been observed to depress the responsiveness of gonads of a fish to injections of fish pituitary extract (Schmidt et al. 1965). Unfortunately, there is little information available on effects of temperature on gametogenesis itself in response to administered hormones.

In the green sunfish (*Lepomis cyanellus*) gonadal recrudescence occurs under the increasing tem-

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peratures and lengthening photoperiods of spring, with active gametogenesis proceeding rapidly to the production of mature gametes (Kaya and Hasler 1972). The combined presence of long photoperiods and elevated temperatures is the effective stimulus to this prespawning maturation of gametes. Both testes and ovaries of this species respond rapidly to stimulation by long photoperiod at 20 C, but do not respond at 10 C (Kaya and Hasler 1972). The objective of the present study is to compare gametogenic responses of *L. cyanellus* treated with pituitary material or androgen while maintained at either of these levels of temperature, to determine whether responses to exogenous hormones would be similarly affected. Positive gametogenic responses to such treatment at low temperature would indicate that the dependence of gonadal recrudescence on elevated temperatures is due primarily to an effect on endogenous hormone production.

Materials and Methods

Experiments were conducted during the winter and early spring of 1969–70. Green sunfish were collected from a pond in Madison, Wisconsin, during late October and maintained in the laboratory until used in experiments. Daily photoperiod of 4 hr (1000–1400, CST) was controlled by an electric timer, and temperatures in the holding tanks, controlled by constant flow of tap water, declined from about 13 C in late October to 7–8 C in January. Under these conditions the gonads remained in the regressed state typical of green sunfish in winter.

Effects of temperature on responses of the gonads to injections of pituitary material (experiment 1) and testosterone propionate (experiment 2) were tested at 10 and 21 C. These temperatures approximate those experienced by the natural population of fish in spring, before and during the period of rapid gonadal development. Fish selected for an experiment were introduced into four 320-liter treatment aquaria at the same temperature as the holding tanks. Temperatures in aquaria of all but the low temperature treatments were allowed to rise to ambient room level and remained at 21±2 C through both experiments. The low temperature aquaria were maintained at 10±.5 C by submersed cooling coils.

Each treatment aquarium was enclosed in a light-tight enclosure and illuminated by two 15-watt, daylight-type, fluorescent lamps. Daily photoperiod remained at 4 hr, as in the holding tanks. Under such abbreviated photoperiod gonads of this species remain in the quiescent winter condition independently of temperature (Kaya and Hasler 1972).

Injections of .05-ml pituitary preparation or 1.0-mg testosterone propionate (Nutritional Biochemicals) dissolved in .025-ml corn oil were administered intraperitoneally on alternate days. Preparation of acetone-dried, powdered carp pituitaries for injection was similar to the procedure described by Clemens and Grant (1964) for preparing a crude "aqueous extract" of whole glands. Amounts of saline solution and powdered glands were measured so that the individual dosage of 2.0 mg was "extracted" in .05 ml. Specimens receiving pituitary material averaged 11.4 g (SD 2.6) in weight, and those receiving testosterone propionate averaged 9.2 g (SD 2.2). Two control groups were used for each experiment, with one group receiving injections of either saline or oil and the other group receiving no injections. Fish were taken for autopsy from each group on each sampling day, 1 or 2 days after the last injections.

Responses of gonads were assessed by two criteria: gonad size, expressed as gonosomatic index (gonad weight × 100/fish weight), and histological evaluation of gametogenic activity. After each gonad was weighed, a midtransverse portion was excised, fixed in Bouin's solution, and prepared for histological study through standard paraffin methods of tissue processing, sectioning at 6–8 μ, and staining with hematoxylin and eosin. Gametogenic activity in experimental specimens was compared with stages of activity that occur during the natural progress of an annual cycle. Spermatogenetic responses were classified into six histological stages (modified after Lofts et al. 1966), summarized as follows: I, Primary spermatogonia predominate, small cysts of secondary spermatogonia present. Advanced stages absent, except for residual spermatozoa in lumina; II, Cysts of secondary spermatogonia predominate and larger in size, advanced stages absent; III, Numerous large cysts of secondary spermatogonia and primary spermatocytes; IV, Cysts of all spermatogenetic stages present, including cysts of spermatozoa; V, Cysts of all spermatogenetic stages present, lumina filled with spermatozoa; VI, Lumina filled with spermatozoa, but exhaustion of spermatogenetic cysts has occurred leaving only residual spermatogonia and Sertoli cells along the walls of the lobules.

The state of maturity of the most advanced class of oocytes present in an ovary was used as an index of ovarian development. Oocyte maturation was divided into six histological stages (modified after Braekevelt and McMillan 1967), summarized as follows: Class I oocytes, 10–120 μ, homogeneous, strongly basophilic cytoplasm; Class II oocytes, 80–200 μ, less basophilic cytoplasm with "frothy" appearance; Class III oocytes, 110–300 μ, primary yolk vesicles in cytoplasm; Class IV oocytes, 210–400 μ, small globules of secondary yolk around periphery of cytoplasm; Class V oocytes, 300–900 μ, larger globules of secondary yolk fill cytoplasm; Class VI oocytes, 750–1100 μ, secondary yolk globules coalesced into large masses that fill cytoplasm.

Results

The temperature at which *L. cyanellus* were maintained during treatment had a very important effect on the responses of their gonads to injected carp pituitary material. Mean gonsomatic indexes of males maintained at 21 C increased rapidly and were much higher than those of the other treatments at all three sampling dates (Fig. 1A). Specimens examined after 1 week of treatment were all at spermatogenetic Stage III (Table 1), and those

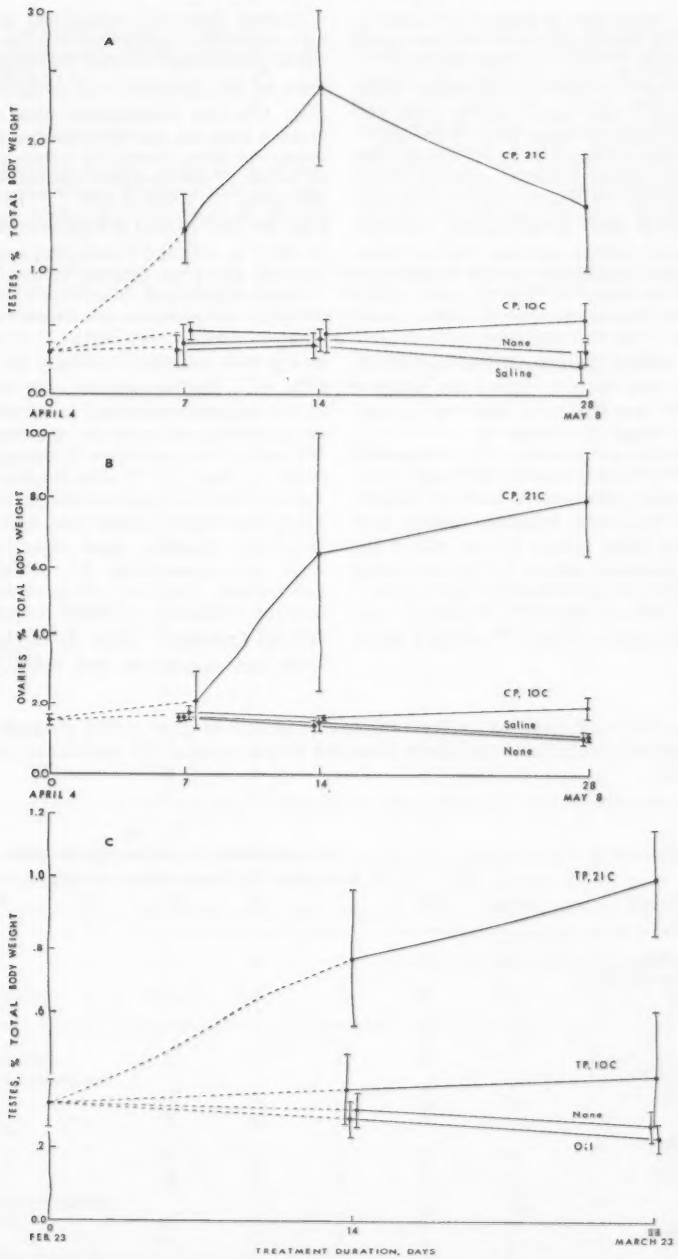

FIG. 1. Effect of temperature on responses of (A) testes and (B) ovaries of green sunfish (*Lepomis cyanellus*) to intraperitoneal injections of preparation of carp pituitary glands (CP), and (C) testes to intraperitoneal injections of testosterone propionate (TP). Each point is a mean ± standard deviation. Numbers of specimens are given in Tables 1–3. All fish maintained at 4-hr daily photoperiod. "None" refers to uninjected controls, "Oil" refers to oil-injected controls, "Saline" refers to saline-injected controls.

examined after 2 weeks were at Stages IV, V, and VI. After 4 weeks of treatment, three of four males had testes at Stage VI and the other at Stage V. Thus, the decrease in mean gonosomatic index between the second and fourth weeks (Fig. 1A) may reflect regression of these Stage VI testes.

In contrast, testes of males at 10 C showed little response to injections of pituitary material. Mean gonosomatic indexes were slightly higher than those of the uninjected and saline-injected controls (Fig. 1A); however, spermatogenesis was not stimulated and the testes remained at the winter condition of Stages I and II (Table 1), histologically similar to those of the original specimens. Mean gonosomatic indexes of the two control groups at 21 C also remained similar to the original condition (Fig. 1A) and most testes remained at Stages I and II, although four fish from these two groups did advance to Stage III (Table 1).

Ovaries of females maintained at 21 C responded to treatment with pituitary material with rapid vitellogenesis. The mean gonosomatic index of females sacrificed from this group increased slightly over that of the other three groups by the end of the first week, and increased greatly during the second week (Fig. 1B). Oocytes progressed to Class IV and V within the first week of treatment, to Class V and VI within 2 weeks, and to Class VI within 4 weeks (Table 2).

Ovaries from fish maintained at 10 C showed little response to treatment with pituitary material. Mean gonosomatic indexes were slightly higher than those of the uninjected and saline-injected controls (Fig. 1B), but vitellogenesis was not stimulated. Ovaries from the low temperature, pituitary-treated group remained similar in histological appearance to those of the original specimens, with oocytes remaining at Class I and II (Table 2). Ovaries from the two control groups also remained similar in size (Fig. 1B) and histological appearance (Table 2) to those of the original specimens.

Temperature had a similar effect on response of testes to injections of testosterone propionate. Testes of males maintained at 21 C increased greatly in size with androgen treatment for 2 and 4 weeks (Fig. 1C). Spermatogenesis was stimulated, with all five specimens sacrificed at the end of the second week having advanced to spermatogenetic Stage III, and all six specimens at the end of the fourth week to Stage IV (Table 3). Mean gonosomatic indexes of the low temperature, testosterone-injected group were slightly higher than those of the controls (Fig. 1C); however, these slight increases in size were not accompanied by stimulation of spermatogenesis. Testes of all specimens at low temperature remained at Stages I and II, as in the original specimens (Table 3). Testes of specimens from both uninjected and saline-injected control

TABLE 1. Effect of temperature on spermatogenetic responses of green sunfish (*Lepomis cyanellus*) to intraperitoneal injections of preparation from carp pituitary glands. Fish maintained at 4-hr daily photoperiod.

Treatment	Days	No.	No. fish advanced to spermatogenetic stage					
			I	II	III	IV	V	VI
Initial condition	0	5	1	4	–	–	–	–
Controls, no injections (21±2 C)	7	4	1	3	–	–	–	–
	13	4	–	3	1	–	–	–
	27	3	–	3	–	–	–	–
Controls, saline (21±2 C)	7	4	1	1	2	–	–	–
	13	4	1	2	1	–	–	–
	27	4	–	4	–	–	–	–
Carp pituitary (10±.5 C)	7	4	–	4	–	–	–	–
	13	4	–	4	–	–	–	–
	27	4	–	4	–	–	–	–
Carp pituitary (21±2 C)	7	4	–	–	4	–	–	–
	13	3	–	–	–	1	1	1
	27	4	–	–	–	–	1	3

groups at 21 C remained similar in size (Fig. 1C) and histological appearance (Table 3) to those of the original specimens.

Discussion

The results demonstrate that, when *L. cyanellus* with gonads in regressed winter condition are maintained under abbreviated photoperiod (4 hr) at 21 C, both testes and ovaries remain quiescent; however, when a crude preparation of carp pituitary material is administered to fish maintained under the same conditions, rapid spermatogenesis and vitellogenesis ensues, producing histologically mature gametes within a 2–4 week period. Specimens maintained at the same temperature, but under long photoperiod (15 hr) can be expected to similarly undergo rapid maturation of gametes (Kaya and

TABLE 2. Effect of temperature on responses of ovaries of green sunfish to intraperitoneal injections of preparation from carp pituitary glands. Fish maintained at 4-hr daily photoperiod.

Treatment	Days	No.	No. fish with oocytes advanced to class					
			I	II	III	IV	V	VI
Initial condition	0	5	–	2	3	–	–	–
Controls, no injections (21±2 C)	7	4	–	1	3	–	–	–
	13	3	–	1	2	–	–	–
	27	4	–	2	2	–	–	–
Controls, saline (21±2 C)	7	4	–	–	4	–	–	–
	13	4	–	–	3	1	–	–
	27	4	–	2	2	–	–	–
Carp pituitary (10±.5 C)	7	4	–	3	1	–	–	–
	13	3	–	1	2	–	–	–
	27	4	–	–	4	–	–	–
Carp pituitary (21±2 C)	7	4	–	–	–	2	2	–
	13	4	–	–	–	–	2	2
	27	4	–	–	–	–	–	4

TABLE 3. Effect of temperature on spermatogenetic responses of green sunfish to intraperitoneal injections of testosterone propionate. Fish maintained at 4-hr daily photoperiod.

Treatment	Days	No.	No. fish advanced to spermatogenetic stage					
			I	II	III	IV	V	VI
Initial condition	0	5	1	4	–	–	–	–
Controls, no injections (21±2 C)	14	6	1	5	–	–	–	–
	28	5	1	4	–	–	–	–
Controls, oil (21±2 C)	14	6	3	3	–	–	–	–
	28	6	2	4	–	–	–	–
Testosterone propionate (10±.5 C)	14	5	2	3	–	–	–	–
	28	6	2	4	–	–	–	–
Testosterone propionate (21±2 C)	14	5	–	–	5	–	–	–
	28	6	–	–	–	6	–	–

Hasler 1972). These observations are consistent with the generally accepted concept that the stimulatory effect of long photoperiod on the gonads is mediated through the pituitary gland. Testes also respond to testosterone propionate by undergoing spermatogenesis, in accordance with the suggestion that such stimulation of spermatogenesis by pituitary hormones may be indirect through stimulation of endogenous androgen production (Lofts et al. 1966; Sundararaj and Nayyar 1967).

Studies of fish maintained under controlled environments have indicated that temperature can also be an important regulator of gametogenesis in certain species of fishes, in some instances with apparently more influence than photoperiod. The literature has been recently reviewed by Schwassmann (1971) and De Vlaming (1972a). There have been very few studies of effects of temperature on gonadal responses to administered hormones, and there is thus little evidence at present on possible physiological relations between temperature and maturation of gametes.

A recent study by Wiebe (1969) supported the earlier suggestion of Atz (1957) that effects of temperature may be mediated through the gonadotropic activity of the pituitary gland. Injections of mammalian LH (luteinizing hormone) stimulated spermatogenesis in shiner perch (*Cymatogaster aggregata*) maintained at 10 C. Stimulation of spermatogenesis in uninjected *C. aggregata* requires elevated temperature (20 C) in addition to long photoperiod (Wiebe 1968). There was no concurrent treatment with LH at higher temperature, however, so the possibility that temperature might also modify the responsiveness of the testes to the administered hormone was apparently not tested.

Modification by temperature of responsiveness of testes of rainbow trout (*Salmo gairdneri*) to administered hormone has been reported by Schmidt et al. (1965). Testes of immature trout maintained at either 5 or 15 C increased in size in response to injections of partially purified extract from salmon pituitary glands, but the higher temperature produced greater enlargements. In contrast, Ahsan (1966c) observed that testes of hypophysectomized lake chub (*Couesius plumbeus*) injected with mammalian LH or extract from salmon pituitary glands were larger at low temperature (9 C) than at high temperature (19 C) during June, July, and August. However, these differences in testis sizes were not accompanied by any consistent histological differences in spermatogenetic responses. These results seem in contradiction to the relation between temperature and the natural testicular cycle of *C. plumbeus*, in which maturation occurs during spring, under increasing water temperature (Ahsan 1966a). However, the experiments began in May on previously hypophysectomized fish and, according to a previous paper by Ahsan (1966b), high temperatures of 19 C applied at this time of year to intact males of this species will induce a rapid regression of testes. Thus, this study with administered LH and pituitary extract appears to have involved effects on regressing rather than maturing testes.

Temperature effects on the regressive phase of an annual sexual cycle were recently investigated by De Vlaming (1972b). The species he studied, the estuarine gobiid fish *Gillichthys mirabilis*, can be induced to undergo gonadal regression by the application of high temperature (27 C). His experiments indicate that high temperatures decrease the responsiveness of the gonads of this species to human chorionic gonadotropin (HGG), LH, and testosterone propionate, but that spermatogenesis can be stimulated by injections of preparations from either homoplastic or salmon pituitary glands in spite of high temperature. De Vlaming concludes that effects on both gonadotropin secretion by the pituitary gland and ability of the gonads to respond to hormone stimulation appear to be involved in gonadal regression induced in *G. mirabilis* by high temperature.

Results of the present study demonstrate that responsiveness of prespawning testes and ovaries of *L. cyanellus* to pituitary hormones is markedly influenced by temperature. Gonads in quiescent, regressed, winter condition respond to administration of carp pituitary material with rapid spermatogenesis and vitellogenesis when fish are maintained at 21 C, but remain quiescent at 10 C. This absence of observable responses at low temperature, in contrast to at least partial responses reported in the previously mentioned studies by Schmidt et al. (1965) and Wiebe (1969), may be due to species differences in temperature at which gametogenesis can proceed. A previous study has indicated that low temperature (10 C) effectively prevents any gametogenic response in *L. cyanellus* to long photoperiod (Kaya and Hasler 1972). However, spermatogenesis does proceed under long photoperiod at low temperature (10 C) in *C. aggregata*, although at a slower rate than at higher temperature (Wiebe 1968). Because *S. gairdneri* is a species known to require colder temperatures (below 13 C) for successful reproduction (McCrimmon 1971), gametogenesis can probably occur at low temperatures in this species also.

There is also the possibility that temperature may affect spermatogenetic activity of the testes through an influence on either androgen production or ability of the germ cells to respond to androgen

stimulation. Results of the present study indicate that responsiveness of testes of *L. cyanellus* to testosterone propionate is also markedly affected by temperature. Spermatogenesis is stimulated in fish injected with this androgen and maintained at 21 C, but not in those injected and maintained at 10 C. According to an unpublished study cited by Bullough (1942), testes of the minnow *Phoxinus* also do not respond to injections of testosterone propionate at low temperature (9 C) but respond by undergoing spermatogenesis at higher temperatures. Further details are not available because the study was apparently never published.

In the present study, although low temperature prevented vitellogenesis in response to pituitary material, and postspermatogonial spermatogenesis in response to either pituitary material or testosterone propionate, gonosomatic indexes of injected fish at low temperature were consistently slightly higher than those of either injected or uninjected controls at higher temperature. The methods employed in this study for analyzing gonadal responses do not provide any information explaining these slightly greater gonad sizes. I can only speculate that this may be the result of increased numbers of spermatogonia or primary oocytes, although there were no apparent increases in mitotic figures in these gonads, or that the administered hormone preparations induced some increase in water content of the gonads, as observed in other species of fish by Clemens and Grant (1964) and De Vlaming (1972b).

In summary, the results do not permit any conclusions as to whether low temperatures act at the level of the brain–pituitary system in this species by inhibiting gonadotropin production, but the results do indicate that the response of the gonads to gonadotropin is inhibited by low temperature and favored by warm temperature. Similarly, whether endogenous steroidogenesis is affected by temperature cannot be determined by this study, but the results do indicate that the stimulatory effect of androgen on spermatogenesis can be inhibited in this species by low temperature. With low temperatures thus imposing an effective inhibition on the gonads, and possibly also on the pituitary gland, the onset of warmer temperatures in the spring is an important prerequisite to the rapid gonadal development that occurs in both sexes at this time of the year.

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AHSAN, S. N. 1966a. Cyclical changes in the testicular activity of the lake chub, *Couesius plumbeus* (Agassiz). Can. J. Zool. 44: 149–159.

1966b. Effects of temperature and light on the cyclical changes in the spermatogenetic activity of the lake chub, *Couesius plumbeus* (Agassiz). Can. J. Zool. 44: 161–172.

1966c. Effects of gonadotropic hormones on male hypophysectomized lake chub, *Couesius plumbeus*. Can. J. Zool. 44: 703–717.

ATZ, J. W. 1957. The relation of the pituitary to reproduction in fishes, p. 178–269. *In* G. E. Pickford and J. W. Atz, The physiology of the pituitary gland of fishes. New York Zoological Society, New York, N.Y.

BAGGERMAN, B. 1972. Photoperiodic responses in the stickleback and their control by a daily rhythm of photosensitivity. Gen. Comp. Endocrinol. Suppl. 3: 466–476.

BRAEKEVELT, C. R., AND D. B. MCMILLAN. 1967. Cyclic changes in the ovary of the brook stickleback, *Eucalia inconstans* (Kirtland). J. Morphol. 123: 373–396.

BULLOUGH, W. S. 1942. Gametogenesis and some endocrine factors affecting it in the adult minnow (*Phoxinus laevis* L.). J. Endocrinol. 3: 211–219.

CLEMENS, H. P., AND F. B. GRANT. 1964. Gonadal hydration of carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) after injections of pituitary extracts. Zoologica (New York) 49: 193–210.

DE VLAMING, V. L. 1972a. Environmental control of teleost reproductive cycles: a brief review. J. Fish. Biol. 4: 131–140.

1972b. The role of the endocrine system in temperature-controlled reproductive cycling in the estuarine gobiid fish, *Gillichthys mirabilis*. Comp. Biochem. Physiol. 41A: 697–713.

HOAR, W. S. 1969. Reproduction, p. 1–72. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology, Vol. III. Academic Press, New York, N.Y.

KAYA, C. M., AND A. D. HASLER. 1972. Photoperiod and temperature effects on the gonads of green sunfish, *Lepomis cyanellus* (Rafinesque), during the quiescent, winter phase of its annual sexual cycle. Trans. Amer. Fish. Soc. 101: 270–275.

LOFTS, B., G. E. PICKFORD, AND J. W. ATZ. 1966. Effects of methyl testosterone on the testes of a hypophysectomized cyprinodont fish, *Fundulus heteroclitus*. Gen. Comp. Endocrinol. 6: 74–88.

1968. The effects of low temperature, and cortisol, on testicular regression in the hypophysectomized cyprinodont fish, *Fundulus heteroclitus*. Biol. Bull. (Woods Hole) 134: 74–86.

MCCRIMMON, H. R. 1971. World distribution of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 28: 663–704.

SCHMIDT, P. J., B. S. MITCHELL, M. SMITH, AND H. TSUYUKI. 1965. Pituitary hormones of the Pacific salmon. I. Response of gonads in immature trout

(*Salmo gairdnerii*) to extracts of pituitary glands from adult Pacific salmon (*Oncorhynchus*). Gen. Comp. Endocrinol. 5: 197–206.

SCHWASSMANN, H. O. 1971. Biological rhythms, p. 371–428. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology, Vol. VI. Academic Press, New York, N.Y.

SUNDARARAJ, B. I., AND S. K. NAYYAR. 1967. Effects of exogenous gonadotropins and gonadal hormones on the testes and seminal vesicles of hypophysectomized catfish, *Heteropneustes fossilis* (Bloch). Gen Comp. Endocrinol. 8: 403–416.

WIEBE, J. 1968. The effects of temperature and day-length on the reproductive physiology of the viviparous seaperch, *Cymatogaster aggregata* Gibbons. Can. J. Zool. 46: 1207–1219.

1969. Endocrine controls of spermatogenesis and oogenesis in the viviparous seaperch *Cymatogaster aggregata* Gibbons. Gen. Comp. Endocrinol. 12: 267–275.

An Established Cell Line from the Atlantic Salmon (*Salmo salar*)

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NICHOLSON, B. L., AND C. BYRNE. 1973. An established cell line from the Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 30: 913–916.

A monolayer culture of fibroblast-like cells was initiated from a trypsin digest of heart, liver, kidney, and spleen tissue of young Atlantic salmon (*Salmo salar*). This culture has been subcultured 81 times over a period of 21 months and appears to be an established cell line, which we have designated AS. Cells of the AS line multiply over a temperature range of 4–28 C with an optimum growth temperature of 20 C. Temperatures in excess of 29 C cause rapid cell death. The AS cells remain viable for periods up to 6 months at 4 C. An absolute plating efficiency of 30% has been determined for AS cells at 20 C. Analysis of the cell cycle of AS cells reveals a generation time (G_t) of 19.5 hr. The duration of the DNA synthetic period (S) is 10.5 hr, while the G_1 and G_2 periods are 1.1 and 7.1 hr, respectively. The duration of mitosis (M) has been calculated to be 0.9 hr with a mitotic index (M.I.) for the culture of 3.3%. Preliminary studies indicate a modal chromosome number of 54. The AS cell line is susceptible to infectious pancreatic necrosis (IPN) virus, infectious hematopoietic necrosis (IHN) virus and the myxovirus of bluegills.

NICHOLSON, B. L., AND C. BYRNE. 1973 An established cell line from the Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 30: 913–916.

Les auteurs ont amorcé une culture à couche monocellulaire de cellules ressemblant à des fibroblastes, à partir d'un digeste trypsique de tissus de coeur, foie, rein et rate de jeunes saumons atlantiques (*Salmo salar*). Après 81 transferts en l'espace de 21 mois, cette culture semble être une lignée cellulaire stable qu'ils nomment AS. Les cellules de la lignée AS se multiplient sur une gamme de température de 4–28 C, avec croissance optimale à 20 C. A des températures dépassant 29 C, les cellules meurent rapidement. Les cellules AS sont viables sur une période de 6 mois à 4 C. On a déterminé une efficacité de plaquage absolu de 30% à 20 C. L'analyse du cycle cellulaire de la lignée AS révèle un temps de génération (G_t) de 19.5 h. La période de synthèse de l'ADN (S) est de 10.5 h, alors que les périodes de G_1 et G_2 sont 1.1 et 7.1 h, respectivement. La durée de la mitose (M) a été établie à 0.9 h, avec indice mitotique (I.M.) de 3.3% pour la culture. Des études préliminaires du nombre chromosomique révèlent la présence d'un mode à 54. La lignée AS est sensible au virus de la nécrose pancréatique infectieuse (NPI), à celui de la nécrose hématopoïétique infectieuse (NHI) et du myxovirus du crapet à oreilles bleues.

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THE first established cell line of poikilothermic origin was the RTG-2 line of Wolf and Quimby (1962), which was initiated from normal gonads of fingerling rainbow trout (*Salmo gairdneri*). Subsequently, many other fish cell cultures have been initiated, including blue-striped grunt (*Haemulon flavolineatum*) fin cultures (FGS) (Clem et. al. 1961), an epithelial-like cell culture from the fathead minnow (*Pimephales promelas*) (FHM) (Gravell and Malsberger 1965), and embryonic Pacific salmon cells (Fryer et. al. 1965). The increased recognition of the significance of viral diseases in fish populations coupled with the current interests in fish culture and management demand further development of fish cell cultures for the diagnosis and study of fish viruses.

This report describes an established line of fibroblast-like cells from tissues of the Atlantic salmon (*Salmo salar*). The cell line, designated AS, has been subcultured 81 times since its initiation in February 1971.

Materials and Methods

METHOD OF ESTABLISHMENT

Atlantic salmon parr were kindly provided by R. Dexter, Craig Brook National Fish Hatchery, East

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Orland, Me. USA. One dozen fish were surface sterilized with .13% Zephiran (Winthrop Laboratories, N.Y.) and rinsed with 95% ethyl alcohol. Heart, liver, kidneys, and spleens were removed aseptically, minced, washed in Earle's balanced salts solution (EBSS), and digested in trypsin. The digestion mixture consisted of .25% trypsin and 2.5% fetal bovine serum in *p*H 7.2 phosphate buffered saline (PBS). Trypsinization was accomplished at 20 C in a fluted erlenmeyer flask on a magnetic stirrer using 10 volumes of digestion mixture for each gram of tissue. Cells obtained after 30 min were discarded and fresh trypsin mixture added. After 6 hr of trypsinization, the cells were harvested by centrifugation in the cold at 300 *g* for 30 min. The cells were washed twice in EBSS with 2.5% fetal bovine serum and finally suspended in growth medium (GM) consisting of Earle's minimum essential medium (EMEM) (Grand Island Biological Company, Grand Island, N.Y. USA) supplemented with 15% fetal bovine serum and containing 100 I.U. of penicillin, 100 μg of streptomycin, 25 I.U. of mycostatin, and 60 μg of tylocine per ml. The cultures were then seeded in plastic tissue culture flasks at 20 C at a density of approximately 10^6 cells per ml as determined from hemacytometer counts using trypan blue.

Growth Curves

Replicate 25 cm^2 plastic flasks were seeded with AS cells, grown at 20 C for 25 hr, and then transferred to the appropriate incubation temperature. The flasks were observed daily and the time required to reach confluency was noted. At selected intervals, flasks were removed and the medium decanted. One ml of PBS was added, the cells scraped off, and the cell suspension decanted. The flasks were then washed once with 1 ml of PBS, which was combined with the first cell suspension and frozen at –85 C. Suspensions were thawed and sonicated for 1 min at 20 kc/sec with a Sonic Dismembrator. The DNA concentration of each cell suspension was then determined by using a modification of the method of Burton (1956). Briefly, to 2 ml of cell suspension were added 2 ml of 10% perchloric acid. After incubation at 70 C for 15 min, 2 ml of diphenylamine (DPA) reagent were added and the incubation continued overnight at 37 C. The cell debris was then removed from the DPA solution by centrifugation at 1000 *g* for 20 min and the supernatent O.D. determined spectrophotometrically at 600 mμ. The DNA concentrations were determined from a standard curve using *Micrococcus lysodeikticus* DNA (Miles Laboratories, Kankakee, Ill. USA).

Analysis of Cell Cycle

Monolayer cultures of AS cells were prepared in Leighton tubes containing coverslips. When the cultures attained 50% confluency or greater, 1 ml of growth medium (GM) from 48–72 hr flask cultures (conditioned medium) and containing 0.5 μc/ml (^{3}H)-methylthymidine (11.2 c/m mole, Schwarz Bioresearch, Orangeburg, N.Y. USA) was added to each tube. After exposure to the label for 15 min at 20 C, the cultures were washed three times with 2 ml of conditioned medium followed by the addition of 1 ml of conditioned medium containing 100-fold excess of unlabeled thymidine. At hourly intervals during the first 10-hr and at 2-hr intervals thereafter, a culture was removed and the coverslips prepared for autoradiography as described previously (Nicholson 1971). Briefly, the coverslips were fixed for 15 min in cold acetic acid: ethanol (1:3), immersed for 10 min in cold 70% ethyl alcohol, air dried, immersed for 30 min in cold 1% perchloric acid, and rinsed for 60 min in running water. After drying, they were coated by immersion in NTB–2 nuclear tract emulsion (Eastman Kodak company, Rochester, N.Y. USA) and following 7 days' exposure at 4 C in the dark were developed in Amidol and stained with 0.5% toluidine blue. The percentage of cells in mitosis and the percentage of mitotic cells with radioactive chromosomes were determined for each culture. The percentage of labeled chromosomes was based on counts of at least 100 mitotic cells per culture.

Plating Efficiency

A suspension of AS cells was prepared from a monolayer culture with 0.02% Versene in phosphate buffered saline (PBS) and the number of viable cells was determined using a trypan blue exclusion technique. Serial 10-fold dilutions of the original cell suspension were made in GM to give suspensions of approximately 3000 and 300 cells per ml. To 10 wells of a plastic microtitration plate were added 0.2 ml of each cell suspension. Following 48 hr of incubation at 20 C, the number of microcolonies of AS cells was determined for each dilution. The mean absolute plating efficiency was then determined based on the number of viable cells counted.

Virus Propagation

The Dry Mills strain of infectious pancreatic necrosis (IPN) virus was propagated in RTG–2 (Wolf and Quimby 1962) cells as described previously (Nicholson 1971). The infectious hematopoietic necrosis (IHN) virus and the myxovirus of bluegills were kindly provided by K. Wolf of the Eastern Fish Disease Laboratory, Kearneysville, West Virginia USA and were propagated in RTG–2 cell cultures.

Results

Establishment of AS Cell Line

Following the trypsinization procedure as described above, viable cells were planted in plastic tissue culture flasks. After 24–hr incubation at 20 C, very few cells had attached. However, some areas of attached viable cells were observed along with several large clumps of cells firmly attached and showing a radial migration of viable cells away from the clump. Fresh medium was added and cultures maintained at 20 C. Incubation was continued for approximately 1 month until cell migration away from the clumps had slowed and cell necrosis was observed in the center of the

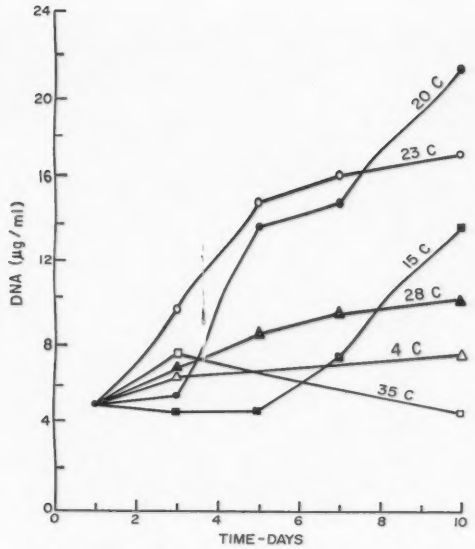


FIG. 1. Growth of Atlantic salmon (AS) cells at various temperatures.

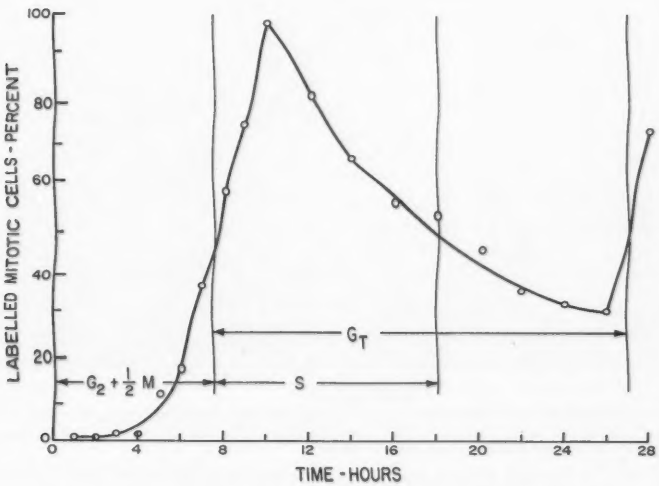
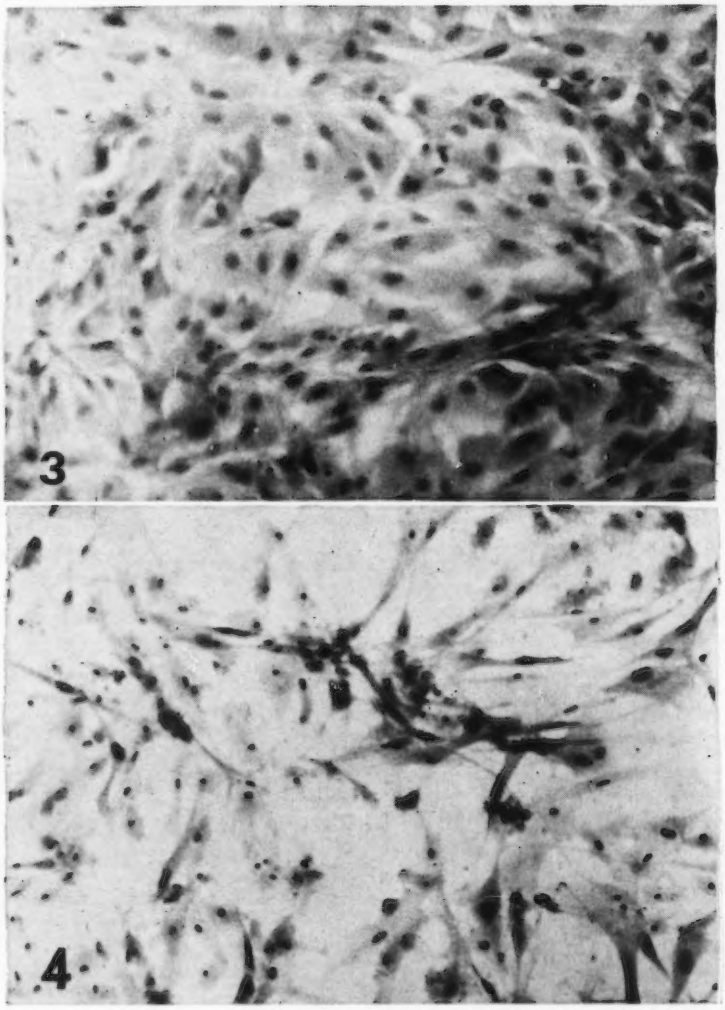


FIG. 2. Cell cycle analysis of Atlantic salmon (AS) cells at 20 C.



FIGURES 3–4

FIG. 3. Normal cells of the Atlantic salmon (AS) cell line. Hematoxylin and eosin. ×140; FIG. 4. Cytopathogenic effect in AS cells infected with infectious pancreatic necrosis (IPN) virus. Hematoxylin and eosin. ×140

clump. At this time, the cells were mechanically dispersed with a sterile glass spreader and fresh media added. After 24 hr, most cells had attached to the flask in a fairly uniform manner with a few small clumps. Confluent cell sheets comprised of fibroblast-like cells were achieved in approximately 2 weeks. From this point on, subculturing the AS cells has been performed with a solution of 0.02% Versene in physiological saline or phosphate buffered saline (PBS). AS cells adhere very firmly to glass or plastic surfaces. To subculture, Versene solution is added in sufficient amounts to completely cover the cell sheet. The cell cultures are then reincubated at 20 C for 20–30 min. Most of the Versene is then decanted and the cells dispersed in the residual Versene by sharply striking the flask several times against the palm of the hand.

Cell Growth

Cell cultures derived from poikilothermic vertebrates will survive and multiply over unusually wide temperature ranges. (Wolf and Quimby 1969). To estimate the optimum growth temperature and the range of permissible growth temperatures, growth curves of AS cells were determined at various temperatures (Fig. 1). After 10 days, cultures incubated at 15, 20, 23, and 28 C were confluent while cultures at 4 C showed little if any change and 35 C temperatures caused complete destruction of the cell sheet. Because DNA has been found to correlate most directly with cell numbers (Kuchler and Merchant 1958), cell growth rates at the different temperatures were determined by measurement of the total concentration of DNA per culture. The maximum rate of cell growth occurred at 20 C with slower growth rates at higher or lower temperatures. Higher temperatures (23 and 28 C) often showed an initially faster growth rate but this invariably leveled off. AS cells did multiply throughout the range of 4–28 C, although little growth occurred at 4 C during the 10-day incubation period. Cultures maintained at 4 C for extended periods of time did eventually reach confluency. Cell death appeared to occur very rapidly when cultures were incubated at temperatures above 30 C. An absolute plating efficiency of 30% was determined for AS cells at 20 C.

Cell Maintenance

AS cells are relatively easy to maintain as monolayer cultures in either plastic or glass vessels. Attempts to grow AS cells in suspension cultures have been unsuccessful. Although AS cells will multiply throughout a relatively wide *p*H range, a *p*H of about 7.4 appears to be optimum. When subcultured at a ratio of 1:2, confluency is usually reached in 2–4 days at 20 C. For maintenance purposes, AS cultures have been split at a 1:5 ratio from which 2–3 weeks at 20 C is required to reach confluency. It is not necessary to change medium during the growth of AS cells. As with other cell lines from poikilothermic origin (Wolf and Quimby 1969; Gravell and Malsberger 1965), long-term maintenance can be achieved by incubating lightly seeded cultures at 4 C. Cultures of AS cells have been maintained in this manner for as long as 6 months without a medium change or loss of viability. Active growth can be restored simply by incubating the cultures at 20 C for several days.

Some success has been achieved in the preservation of AS cells by freezing at –70 C in 10% glycerol. Recovery of viable cells has been erratic, however, and further work must be performed before freezing can be routinely used as a preservation procedure for AS cells. The AS cell line has now been subcultured 81 times since its initiation in February, 1971. Preliminary studies of AS cells indicate a pseudo diploid chromosome number with a mode of 54 (Willard and Roberts 1972).

Cell Cycle Analysis

The results shown in Fig. 2 illustrate a typical pulse chase analysis of AS cells grown in EMEM supplemented with 15% fetal bovine serum. The cohort of labeled cells was followed through two successive divisions. Measurements of the duration of the periods within the interphase or intermitotic period of the cell cycle were made by determining the percentage of labeled mitoses as a function of time after incorporation of (^{3}H)–methylthymidine. After separation of the daughter cells at telophase, there is first a period or gap (G_1) in which the DNA content is kept constant (at $2n$). Subsequently, a period of active DNA synthesis (S-period) ensues. Then, when duplication is completed and prior to the next division, there is a second gap or period (G_2) in which the DNA content is kept constant (at $4n$). The duration of the AS cell cycle (G_t) and the G_1, S, and G_2 periods were calculated from the 50% intercepts on the curve in Fig. 2 according to the method of Graves (1972). The mitotic time (M) was calculated independently using the formula $M = \text{mitotic index (M.I.)} \times G_t/1n2$ (Graves 1972). The time elapsed between the 50% labeling index on the ascending portion of the first and second curves represents the duration of the cell cycle or generation time of AS cells (G_t = 19.5 hr). With a M.I. of 3.3%, a duration of 0.9 hr was calculated for M. The time from zero to the first

50% index represents the duration of (G_2 + 1/2M). Thus, G_2 encompasses 7.1 hr. The length of the S-period of the cell is represented by the time interval between the 50% intercepts of the ascending and descending portions of the first curve (S = 10.5 hr). The G_1 period was obtained from the difference between the generation time (G_t) and the sum of the G_2, S, and M periods (G_1 = 10.5 hr).

VIRUS SUSCEPTIBILITY

The AS cell line will support the replication of the infectious pancreatic necrosis (IPN) virus. The IPN virus has been grown to titers of $10^{8.5}TCID_{50}$/ml in AS cells at 20 C. The appearance of cytopathic effects (CPE) (Fig. 3 and 4) in IPN virus infected AS cultures is similar to that in RTG–2 cells except, perhaps, not as severe. That is, the degenerative cells characteristically retain their elongate shape. Virus yields are as high, or higher, in AS cells as in RTG–2 cells. Two other fish viruses, the infectious hematopoietic necrosis (IHN) virus and the myxovirus of bluegills, have also been propagated in cells of the AS line.

Discussion

With the increased recognition of the significance of viral diseases in fish populations, much interest is now being focused on the development of cell culture techniques for the diagnosis and study of fish viruses. The development of the AS cell line described in this report provides a useful tool for the isolation and identification of viruses infecting Atlantic salmon. Although mortalities have occurred in Atlantic salmon populations which might possibly have been caused by a virus infection, no direct evidence has been accumulated that would link a virus with these mortalities. IPN virus has been isolated from Atlantic salmon (MacKelvie and Artsob 1969; Dexter 1972) but, to date, no mortalities in Atlantic salmon can be directly attributed to IPN virus. One reason for the limited knowledge of viruses infecting Atlantic salmon probably is the lack of a suitable method for isolating and identifying viruses from normal and diseased fish. With the development of the AS cell line it is now possible to screen in the laboratory both normal and diseased salmon populations for potentially destructive viruses. In addition, the AS line provides another laboratory host system for the study of the replication of fish viruses of other species. In this report, we have demonstrated that Atlantic salmon cells propagated in vitro are susceptible to IPN virus, IHN virus, and the myxovirus of bluegills. We are currently studying the effect of experimental infection of live fish with these viruses.

Acknowledgments

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BURTON, K. 1956. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62: 315–323.

CLEM, L. W., L. MOEWUS, AND M. M. SIGEL. 1961. Studies with cells from marine fish in tissue culture. Proc. Soc. Exp. Biol. Med. 108: 762–766.

FRYER, J. L., A. YUSHA, AND K. S. PILCHER. 1965. The in vitro cultivation of tissue and cells of Pacific salmon and steelhead trout. Ann. N.Y. Acad. Sci. 126: 566–586.

GRAVELL, M., AND R. G. MALSBERGER. 1965. A permanent cell line from the fathead minnow (*Pimephales promelas*). Ann. N.Y. Acad. Sci. 126: 555–565.

GRAVES, S. A. M. 1972. DNA synthesis in heterokaryons formed by fusion of mammalian cells from different species. Exp. Cell Res. 72: 393–403.

KUCHLER, R. J., AND D. J. Merchant. 1958. Growth of tissue cells in suspension. Univ. Mich. Med. Bull. 24: 200.

MACKELVIE, R. M., AND H. ARTSOB. 1969. Infectious pancreatic necrosis virus in young salmonids of the Canadian Maritime Provinces. J. Fish. Res. Board Can. 26: 3259–3262.

NICHOLSON, B. L. 1971. Macromolecule synthesis in RTG–2 cells following infection with infectious pancreatic necrosis (IPN) virus. J. Gen. Virol. 13: 369–372.

WOLF, K., AND M. C. QUIMBY. 1962. Established eurythermic line of fish cells in vitro. Science 135: 1065–1066.

WOLF, K., AND M. C. QUIMBY. 1969. Fish cell and tissue culture, p. 253–305. *In* W. S. Hoar and D. S. Randall [ed.] Fish physiology. Vol. III. p. 253–305. Academic Press, New York, N.Y.

Some Comparisons in the Thermal Structure of Lakes Wood, Kalamalka, Okanagan, Skaha, and Osoyoos, British Columbia

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BLANTON, J. O. 1973. Some comparisons in the thermal structure of lakes Wood, Kalamalka, Okanagan, Skaha, and Osoyoos, British Columbia. J. Fish. Res. Board Can. 30: 917–925.

The rate of change of heat contents in the lakes and the relative ability of each lake to transfer heat vertically to warm its hypolimnion was investigated. Rates of warming of hypolimnion water ranged from 0.06 C/month in Okanagan to 0.54 C/month in the north basin of Osoyoos. All lakes reached maximum static stability through the thermocline in late August except for Osoyoos (N), which reached its maximum somewhat earlier. Maximum heat content ranged from 18,100 cal/cm^2 in Wood Lake to 33,300 cal/cm^2 in Lake Okanagan. Maximum values of heat content were observed in late August in all lakes.

There is a direct relation between the hypolimnetic warming rates and the maximum observed stability in the thermocline regions of all the lakes. However, the relatively low rate of Lake Wood indicates that there is an external cooling source such as groundwater influx. The late summer increase in volume of the epilimnion in Wood and the corresponding entrainment of nutrients from the hypolimnion to the epilimnion appear to control the amount of production observed at that time.

BLANTON, J. O. 1973. Some comparisons in the thermal structure of lakes Wood, Kalamalka, Okanagan, Skaha, and Osoyoos, British Columbia. J. Fish. Res. Board Can. 30: 917–925.

Nous avons étudié le taux de changement du contenu calorifique des lacs et leur capacité relative de transférer la chaleur verticalement pour le réchauffement de l'hypolimnion. Le taux de réchauffement de l'hypolimnion est le plus lent dans le lac Okanagan (0.06 C/mois) et le plus rapide dans le bassin nord du lac Osoyoos (0.54 C/mois). Tous les lacs atteignent un maximum de stabilité statique dans la thermocline à la fin d'août, sauf le lac Osoyoos (N), qui l'atteint un peu plus tôt. Le contenu calorifique maximal le plus bas est de 18,000 cal/cm^2 dans le lac Wood; le plus élevé est de 33,300 cal/cm^2 dans le lac Okanagan. Dans tous les lacs, les valeurs maximales sont atteintes en août.

Il existe une relation directe entre le taux de réchauffement hypolimnétique et la stabilité maximale de la région de la thermocline dans tous les lacs. Cependant, le taux relativement bas du lac Wood indique la présence d'une source externe de refroidissement, telle un influx d'eau souterraine. L'augmentation du volume de l'épilimnion dans le lac Wood en fin d'été et le transfert correspondant de substances nutritives de l'hypolimnion à l'épilimnion semblent contrôler la production à cette période.

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A large interdisciplinary study of the principal lakes in the Okanagan Basin, British Columbia, was undertaken jointly by Federal and Provincial agencies during 1971, and five of these lakes received detailed limnological attention. The lakes lie in a common drainage area in southern central British Columbia. The outlet of the chain, the Okanagan River, is a tributary within the Columbia River system. It is appropriate to describe the basic findings concerning the thermal structure of these lakes because (1) the five lakes have widely different morphometry, yet are exposed to similar regimes of radiation; and (2), until 1971, no lake in western Canada had a large physical limnological data base; therefore the thermal structure should be of interest to limnologists working in this region of North America.

The year 1971 does not appear to be a typical year climatically (Blanton and Ng 1972), which may make difficult the quantitative extrapolation of the data. Between-lake comparisons are expected to remain valid in a qualitative sense.

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Data and Analytical Methods

Basic morphometric data for the five lakes are summarized in Table 1. The set of data discussed here were obtained on Wood, Kalamalka, Okanagan, Skaha, and the northern basin of Osoyoos[1]. These lakes were monitored six times (Kalamalka, five times) in 1971. The details of this monitoring plan are described by Blanton and Ng (1972) and Williams (1972). The density of station coverage for determining the temperature structure varied from four stations in Wood to 19 stations for Okanagan. The stations were approximately evenly spaced along the longitudinal axes of the lakes. The monitoring cruises began in late April as summer stratification was beginning. The cruises were repeated at monthly intervals, the last cruise occurring near the beginning of October.

The thermal structure has been described from temperature data obtained with bathythermographs (BT) manufactured by Wallace and Tiernan, Inc. These instruments may be read with temperature accuracies of ± 0.1 C and depth accuracies of $\pm 1\%$ of the depth scale. For the 275-meter BT used in the deepest portions of Lake Okanagan, depth is known within ± 3 m. The depth accuracy in general depended upon the local depth. At depths between 60 and 140 m, a BT was selected that gave an accuracy of ± 1.4 m; at depths less than or equal to 60 m, accuracy was better than ± 1 m.

The average temperature profile was calculated for each cruise on each lake. The average depth of the isotherms was determined from the BT traces. The depth averages have been weighted by an area of influence assigned to each station. From the averaged temperature profile, it was possible to summarize the temporal changes in selected temperature ranges. The volumes of these ranges were determined from a hypsometric curve of each lake (Blanton and Ng 1972). The profiles were also used to estimate the volumes of the epilimnion, the metalimnion, and the hypolimnion. The boundary between the three regions was defined by two points on the temperature–depth curve. The first point separates the epilimnion from the metalimnion and is located at the maximum *change* in the vertical temperature gradient above the thermocline; the second point separates the metalimnion from the hypolimnion and corresponds to the maximum change below the thermocline.

Some idea of the spatial deviations from the average within each lake may be gained from plots of isotherms along the longitudinal axes. These plots are available from the author upon request.

The total heat content, H_T, for each lake for a particular cruise was determined from the sum of the heat contents for the epilimnion, H_E; the thermocline region, H_M; and the hypolimnion, H_H. The actual calculation gave the heat content in cal/cm^2, or

$$Q = \frac{c_p \rho}{A_o} \left[V_H(T_H - 4) + V_M(T_M - 4) + V_E(T_E - 4) \right]$$

where c_p is the specific heat of water, ρ is the water density, and A_o is the surface area of the lake. The product, $c_p \rho$, was taken as 1. The maximum value of Q during a given season has been defined by Hutchinson (1957) as the summer heat income.

Results

Temperature Changes

At the time of the first cruise, Lake Wood (Fig. 1a) had no temperatures below 4 C. The heat in the lake increased rapidly with time so that, by the time of the fourth cruise, 20% of its volume had temperatures above 20 C. The average temperature of the hypolimnion in Wood was less than 6 C so that the volume between 4 and 6 C may be taken as representative of the volume of the hypolimnion.

[1]Hereafter referred to as Osoyoos (N).

Table 1. Morphometry of five mainstem lakes in the Okanagan Basin.

Lake	Volume ($10^6 m^3$)	Surface area ($10^6 m^2$)	Depths (*m*) Mean	Depths (*m*) Maximum	Maximum[a] length (*km*)	Maximum[a] width (*km*)	Perimeter[a] (*km*)
Wood[b]	200	9.3	22	34	6.60	1.70	16.7
Kalamalka[b]	1520	25.9	59	142	16.0	2.30	42.4
Okanagan[b]	26200	348	76	242	112.8	5.22	270
Skaha[c]	558	20.1	26	57	11.90	2.40	29.5
Osoyoos (N)[b,d]	204	9.91	21	63	7.55	1.75	20.4
Osoyoos (S)[b,d]	51.5	5.14	10	29	3.00	1.90	12.8

[a]These data were obtained from maps of the Canadian National Topographic System, 1960. Scale 1: 126, 720.
[b]Data compiled from charts of the Fish and Wildlife Branch, Department of Recreation and Conservation, B.C.
[c]Data compiled from a chart by A. M. Thomson.
[d]Osoyoos (N) is the basin north of the highway bridge. Osoyoos (S) is the basin between the highway bridge and the U.S. border.

Stratification was well underway at the time of the second cruise. While there was some evidence of a well-defined epilimnion at Cruise 3, the epilimnion had been virtually wiped out by strong heating just previous to Cruise 4. The lake was strongly stratified with a well-defined epilimnion for the final two cruises. Rapid cooling of the epilimnion occurred between cruises 5 and 6.

One may see marked differences in the thermal structure between Kalamalka (Fig. 1b) and the three shallower lakes in the chain, e.g. 75% of its volume remained at temperatures less than 6 C throughout the field season. During Cruise 5, when temperatures were at a maximum, less than 10% of its volume had temperatures above 20 C. Obviously, Kalamalka had a relatively large and cold hypolimnion compared with that of the shallower lakes. While the deeper portions of the lake began the stratification season with temperatures less than 4 C, the deep water had warmed above 4 C at the time of Cruise 2.

There were no significant differences observed between the eastern and western arms of Kalamalka. The thermocline remained relatively shallow for

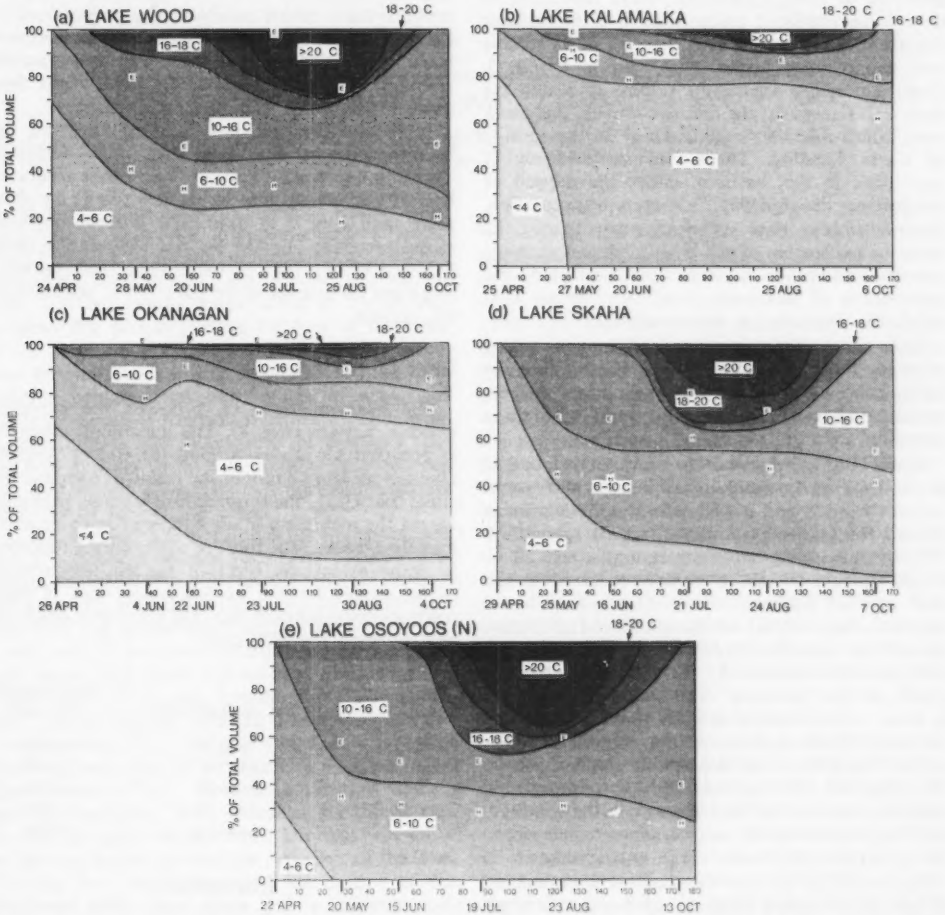

FIG. 1. Volumes associated with given temperature ranges observed in the Okanagan lakes during 1971. The volume scale is cumulative from the bottom of each lake. The median dates of each cruise have been included on the time axes. The E and H symbols mark the approximate position of the epilimnion and hypolimnion, respectively, on the cumulative volume scale.

all cruises. A well-defined mixed layer was found only for Cruise 6, and this layer was confined to less than 25% of the total lake volume.

There was no clear evidence from the temperature data of an influence from Wood on the thermal structure at Station 1 in Kalamalka. Surface temperatures at Station 1 at the northern end of Wood Lake were usually from less than 1 to less than 3 C warmer than those at Station 1 in the southern end of Kalamalka.

Except for its relatively large volume of cold water, Okanagan (Fig. 1c) is quite similar in thermal structure to Lake Kalamalka. More than 65% of its volume remained colder than 6 C throughout the stratification period. Less than 5% of its volume had temperatures above 20 C during Cruise 5. Okanagan had a significant volume of water less than 4 C. Except for the first two cruises, this water was found only in the deep basin in the vicinity of Carrs Landing. There was no evidence of meromixis in this basin in either the oxygen or conductivity data for 1971. During spring circulation, there must have been sufficient energy to mix the water to the bottom of this basin. (All temperatures measured during the monitor cruises were above the temperature of maximum density for the depth at which the temperature was measured.)

Both arms in the north of Okanagan heated relatively faster than other parts, and they became highly stratified in summer. Highest surface temperatures for a given cruise were always found in these arms.

Skaha (Fig. 1d) had no temperatures below 4 C at the time of Cruise 1. Stratification had begun before Cruise 2, and a well-defined epilimnion was present for Cruises 2–6. For Cruise 4 more than 15% of the volume had temperatures above 20 C. Rapid cooling of the epilimnion occurred in the time interval between cruises 5 and 6. Warmer temperatures occurred at the south end of Skaha during the second, third, and fourth cruises, a fact possibly explained by the predominant north winds at the Penticton Airport for this period. A ridge in the southern portion of the lake divides the lake into two basins — a large northern basin and a slightly shallower and smaller southern basin. The ridge did not obviously affect the temperature structure, but bottom temperatures in the southern basin were consistently warmer than temperatures at the same depth in the large northern basin.

Osoyoos (Fig. 1c) had the warmest hypolimnion of the lakes studied (Fig. 4, represents the large northern basin only). At Cruise 1 most of its volume included water above 6 C. Stratification had also begun by the time of the second cruise. At Cruise 5 more than 30% of the lake's volume had temperatures greater than 20 C. The line representing temperatures less than 10 C describes the approximate time rate of change of the hypolimnion volume. The epilimnion was well defined from the second until the last cruise.

Osoyoos is divided into a large northern basin [Osoyoos (N) in this paper], a small Canadian basin, and a large southern basin that extends from within Canada southward beyond the international border. Surface temperatures generally increased from north to south. The effects of the ridges separating the basins were quite evident in the thermal structure, and the thermal differences between these basins became more pronounced as the stratification season progressed. The northern basin was strongly stratified. The hypolimnion warming rate for this basin was more rapid than those of the other lakes (0.54 C/month).

In the smaller Canadian basin of Osoyoos, the bottom temperature regime was quite different. This is not surprising because this basin is separated from the adjoining ones by shallow sills at no place more than 1 or 2 m deep. The waters were well mixed vertically and the bottom temperature cycle followed closely that of the surface. Bottom temperatures were never more than 2–4 C colder than the surface.

Heat Contents

The heat contents, Q, followed the expected seasonal pattern (Fig. 2). The maximum values of Q occurred for cruises around the end of August. One can see from a plot of the mean air temperature curve that Q_{max}, the summer heat income, probably lagged the maximum in air temperature by approximately 2 weeks. The form of the Q curves followed to some extent the form of the air temperature curve, except for the expected slow loss of heat content during the early fall. The maximum observed heat contents for 1971 were:

Lake	Time of observation	Heat content (cal/cm^2)
Okanagan	Aug. 30	33,300
Kalamalka	Aug. 25	25,100
Skaha	Aug. 24	22,200
Osoyoos (N)	Aug. 23	21,900
Wood	Aug. 25	18,100

Discussion

While the lakes were not observed for the entire year, they are most surely dimictic (two circulation periods per year). Wood, Kalamalka, Skaha, and Osoyoos may be further classified as dimictic lakes

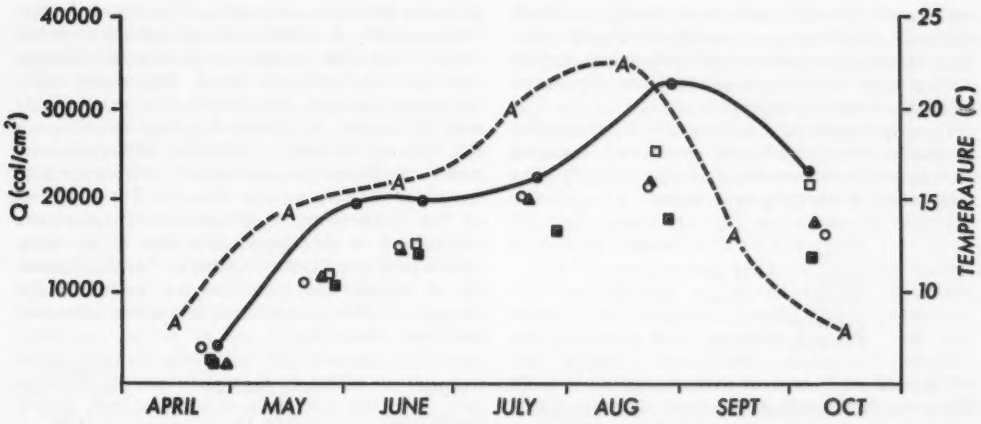

FIG. 2. Heat contents (Q) observed in 1971 for Okanagan (●), Kalamalka (□), Wood (■), Skaha (▲), and Osoyoos (N) (○). "A" represents the monthly mean air temperature in 1971 at Penticton airport.

of the second class since their bottom temperatures are somewhat above 4 C in midsummer (see Hutchinson 1957 for a discussion of thermal classifications). Kalamalka is marginally between first and second class because bottom temperatures in the deepest portions are probably close to 4 C at all times of the year. Lake Okanagan, on the other hand, may be considered a first class lake for the deep portion between the two northern arms and Kelowna. South of Kelowna information on bottom temperatures is too incomplete for certainty, but the lake appears to be a borderline case between first and second class. The two northern arms are definitely of the second class.

Calculated mean temperatures for the epilimnion and hypolimnion of each lake (Fig. 3) show that the epilimnion of Wood heated the most. In general, however, the seasonal pattern of epilimnion temperatures was similar from lake to lake.

All lakes at the time of Cruise 1 were relatively well mixed and there was no real distinction between epilimnion and hypolimnion. Kalamalka had a large zone in midlake where temperatures were less than 4 C. There appeared to be a "thermal bar" on both ends of the lake (see Rodgers 1965). The southern "bar" (between stations 4 and 5) and the northern "bar" (between stations 2 and 4) were probably slices of one bar that was more or less continuous around the periphery of the lake. The bar is a manifestation of the heating of the shallower areas near shore and is evidence that Kalamalka may have been fully circulating vertically in the central area of the lake. Thermal bars can be associated with the beginning of the stratification season in spring and in winter.

At the time of Cruise 2 stratification had begun. Kalamalka and Okanagan had poorly defined epilimnia, while Wood, Skaha, and Osoyoos had well-mixed epilimnia down to 5, 7, and 10 m, respectively. Between Cruise 3 and 4, at the time when the monthly mean temperature increased greatly (Fig. 2), the well-mixed character of the epilimnia became confused. There was little evidence of wind mixing over that period, and Wood virtually lost its well-defined epilimnion. (Many lakes reflect this period of strong surface heating when epilimnia become ill-defined.) During late August and through September all epilimnia became well mixed and increased in volume.

Peak epilimnion temperatures (Fig. 3) do not reflect the times of maximum lake temperature. Highest daily average temperatures observed from recording thermographs in the lakes indicated that each reached its maximum temperature between the end of July and the middle of August, i.e. between cruises 4 and 5. This period coincided with the approximate time of the highest daily mean air temperature at the Penticton Airport.

The epilimnion temperatures in Okanagan were relatively low in the southern portion between Squally Point and Penticton when compared to those north of Squally Point. There is some evidence of upwelling from an array of moored thermographs in the southern end, but upwelling only occurred for about 10% of the time between May 12 and September 28, 1971. I suspect that the top of the shallow thermocline has been eroded and the surface water cooled by mixing. The fetch from Squally Point to the beach at Penticton is sufficiently long so that significant amounts of turbulence could

be injected into the epilimnion during northerly winds. In other words, one might expect the maximum thermocline erosion and subsequent surface cooling to be near the southern end where exposure to north winds is maximum.

The hypolimnia of the five main lakes warmed at much slower rates and continued warming throughout the observation period (Fig. 3). The magnitudes of warming rates were:

Osoyoos (N)	0.54	C/month
Skaha	0.37	"
Wood	0.26	"
Kalamalka	0.18	"
Okanagan	0.06	"

The observed warming rates appear closely linked to the maximum observed stability (E) of the lakes. E most accurately describes stratification and may be defined as $\frac{g}{\rho}\frac{\delta\rho}{\delta z}$ where $\frac{\delta\rho}{\delta z}$ is the change in water density per unit depth, z; and g is the acceleration of gravity. The zone of maximum stability in a lake is found in the thermocline region. For this region the maximum observed stability has been calculated during the 1971 monitoring season. Maximum observed stability was at the time of Cruise 5 except for Osoyoos (N). Density changes through the thermocline are based on lake-wide averages of epilimnion and hypolimnion temperatures (Fig. 3). The thickness of the thermocline is derived from hypsometric curves and is the depth difference found when subtracting the depth represented by the volume of the hypolimnion from that represented by the volume of the epilimnion. Maximum observed stabilities were:

Lake	Date observed	Stability ($sec^{-2} \times 10^6$)
Osoyoos (N)	July 19	2120
Skaha	Aug. 24	1750
Wood	Aug. 25	1440
Kalamalka	Aug. 25	1090
Okanagan	Aug. 30	850

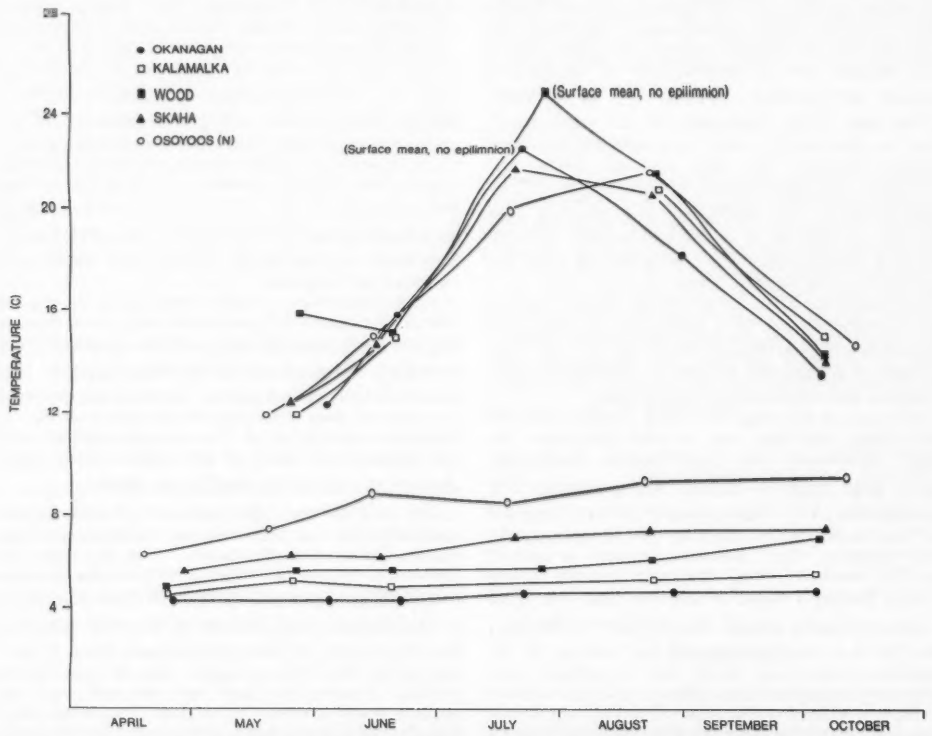

FIG. 3. Lake-wide mean temperatures for the epilimnion and hypolimnion in 1971.

The close link between E_{max} and the warming of the hypolimnion is apparent in Fig. 4. (Remember that a deep lake such as Okanagan transfers much more heat to its hypolimnion than that transferred in shallower lakes, but its relatively enormous volume prevents much increase in temperature.) The shallowest three lakes had the highest stability observed and the highest rate of warming. This result is quite reasonable because even a small amount of heat transfer would be sufficient to raise the temperature of a relatively small hypolimnion volume. Moreover, the relatively small vertical extent of the hypolimnion in the three shallow lakes probably limits the energy of turbulent motion in the hypolimnion. In other words, there is insufficient hypolimnetic turbulence to erode the thermocline from below to the same degree that is possible in a lake with a greater vertical dimension in its hypolimnion (Mortimer 1942).

The rate of heat transferred to the hypolimnia of the five lakes has been estimated by plotting each hypolimnion's heat content in cal/cm^{-2} versus time. A best fit regression line to these values over the time interval from late June to early October yields only one significant figure, but the results are enlightening:

Lake	Heat transfer (*cal/cm²/day*)
Wood	−6
Kalamalka	+30
Okanagan	+60
Skaha	+20
Osoyoos (N)	+30

The rates of increase from cruise to cruise were highly variable for each lake, therefore no coefficients of vertical eddy diffusivity through the thermoclines are tabulated. Sweers (1970) found similar extreme variability for Lake Ontario, and the interpretation of diffusivity coefficients under these conditions becomes quite ambiguous. Nevertheless, the transfer rates in the table above indicate a puzzling thermal behavior for Wood. The sign (negative) of the heating rate seems certain although its magnitude may be somewhat questionable. Any vertical thermal diffusivity for Wood calculated from these data would be quite meaningless. The negative transfer rate is awkward to interpret in a physical sense unless one assumes that the heat transferred is used for something other than raising the heat content per unit area of the hypolimnion as defined here.

The curious thermal behavior of Wood during 1971 is indicated by several factors. First, the warming rate (degrees C/month) of the hypolimnion was somewhat low compared with the other two lakes of similar morphometry. Second, the hypolimnion actually appeared to lose heat during the stratification season. (Even the most pessimistic estimate would indicate that the gain, if any, was an order of magnitude or more below the other lakes in the chain.) Third, the maximum heat content of the lake (Fig. 5) appeared too low for a lake with its volume. Four of the five Okanagan lakes exhibit a good correlation between their maximum observed heat income (summer heat income) and (volume)$^{\frac{1}{3}}$. Wood, however, is below the curve. Morphometric control of annual heat budgets in lakes has been demonstrated by Gorham (1964) for a wide geographical range of lakes in the northern hemisphere. I feel that summer heat income for each of the Okanagan lakes may be a more physically realistic parameter than annual heat income because the different amounts of ice cover within the five lakes greatly affect the annual budget. I would speculate that the summer heat income

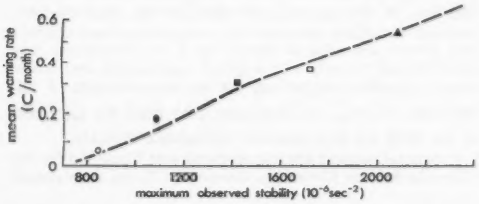

FIG. 4. Rate of hypolimnetic warming versus maximum observed stability. Symbols are defined in Fig. 2.

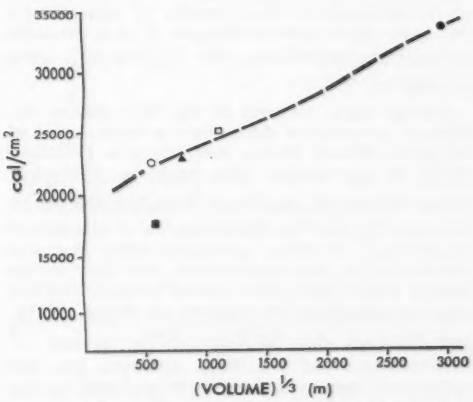

FIG. 5. Maximum heat content in cal/cm^2 versus (volume)$^{\frac{1}{3}}$. Line is fitted by eye. Symbols are defined in Fig. 2.

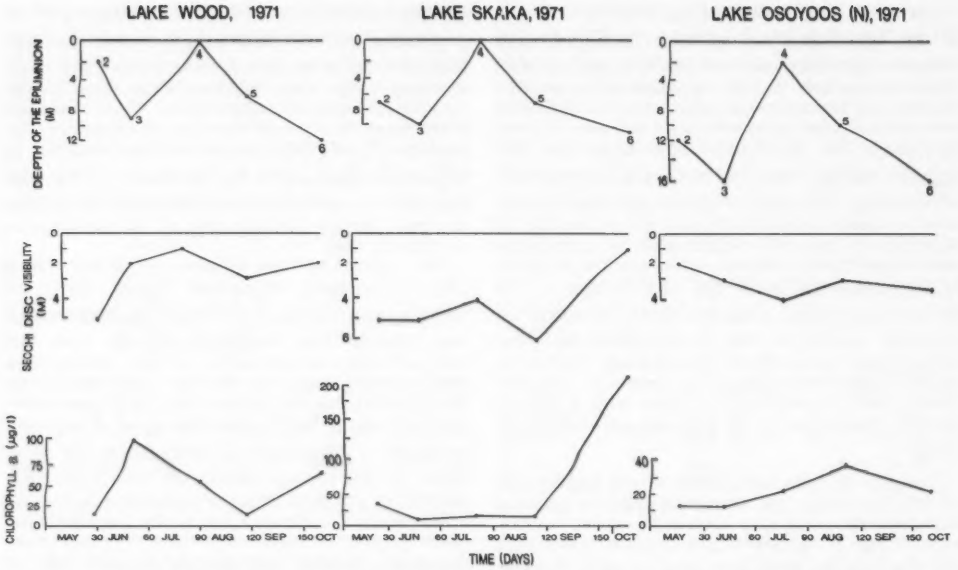
LAKE WOOD, 1971
LAKE SKAHA, 1971
LAKE OSOYOOS (N), 1971
DEPTH OF THE EPILIMNION (M)
SECCHI DISC VISIBILITY (M)
CHLOROPHYLL a (µg/l)
MAY 30 JUN 60 JUL 90 AUG 120 SEP 150 OCT
TIME (DAYS)

FIG. 6. Comparison of chlorophyll-*a* measurements at a 1-m depth with the depth of the epilimnion and with the depth of visibility of the secchi disc.

should offer a more convincing correlation with a morphometric parameter than does annual heat budget for any group of lakes exposed to a similar climate. The findings of Schindler (1971) for lakes in western Ontario appear to confirm this.

The above three findings lead to the tentative conclusion that a large groundwater influx may act to diminish the heat content of Lake Wood. No other agent can be thought of as a plausible explanation; nevertheless, the conclusion is quite speculative.

During some portions of the field season significant increases in chlorophyll *a* were measured in Lakes Wood, Skaha, and Osoyoos (Williams 1972). In late summer these peaks in chlorophyll *a* were preceded by periods of increasing epilimnion volumes (Fig. 6). An epilimnion that is in a period of growth is, in effect, entraining water that was previously in the hypolimnion and thermocline region. When these water masses contain relatively high concentrations of nutrients, as Wood, Skaha, and Osoyoos did (Williams 1972), periods of entrainment could mix these nutrients into the epilimnion, thereby making them available for use in primary production. The amount of entrainment over a given period is dependent upon the turbulent energy induced into the epilimnion by the wind and upon the stability of the water across the thermocline region. Figure 6 suggests that entrainment plays perhaps a dominant role in the late summer production increases in Wood and probably exerts some influence on production in Skaha. The influence of entrainment upon production in Osoyoos (N) is not clear. Low external loading during that period and falling epilimnion temperatures probably rule out those factors as possible stimulants to productivity for Wood and Skaha.

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BLANTON, J. O., AND H. Y. F. NG. 1972. The physical limnology of the mainstem lakes in the Okanagan Basin, British Columbia (Vol. 1: Results). Canada

Centre for Inland Waters, Burlington, Ontario. MS Rep. 34 p.

GORHAM, E. 1964. Morphometric control of annual heat budgets in temperate lakes. *Limnol. Oceanogr.* 9: 525–529.

HUTCHINSON, G. E. 1957. A treatise on limnology. Volume 1: Geography, physics and chemistry. John Wiley & Sons, New York, N.Y. 1015 p.

MORTIMER, C. H. 1942. The exchange of dissolved substances between mud and water in lakes. J. Ecol. 30: 147–201.

RODGERS, G. K. 1965. The thermal bar in the Laurentian Great Lakes. Proc. 8th Conf. Great Lakes Res., Univ. Michigan, Great Lakes Res. Div., Publ. 13: 358–363.

SCHINDLER, D. W. 1971. Light, temperature and oxygen regimes of selected lakes in the Experimental Lakes Area, Northwestern Ontario. J. Fish. Res. Board Can. 28: 157–169.

SWEERS, H. E. 1970. Vertical diffusivity coefficient in a thermocline. Limnol. Oceanogr. 15: 273–280.

WILLIAMS, D. J. 1972. General limnology of the mainstem lakes in the Okanagan Valley, B.C. Canada Centre for Inland Waters, Burlington, Ontario. MS Rep. 13 p.

Identification of Supernatant and Mitochondrial Isozymes of Malate Dehydrogenase on Electropherograms Applied to the Taxonomic Discrimination of Walleye (*Stizostedion vitreum vitreum*), Sauger (*S. canadense*), and Suspected Interspecific Hybrid Fishes

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CLAYTON, J. W., R. E. K. HARRIS, AND D. N. TRETIAK. 1973. Identification of supernatant and mitochondrial isozymes of malate dehydrogenase on electropherograms applied to the taxonomic discrimination of walleye (*Stizostedion vitreum vitreum*), sauger (*S. canadense*), and suspected interspecific hybrid fishes. J. Fish. Res. Board Can. 30: 927–938.

Walleye (*Stizostedion vitreum vitreum*) and sauger (*S. canadense*) commonly yield four isozymes of malate dehydrogenase (MDH) in electropherograms of white muscle extracts from individual homozygous fish. The heat stability of each isozyme as well as reactivity with nicotinamide-adenine dinucleotide (NAD) and NAD analogues have been investigated by measuring the density of each isozyme directly on the electropherograms, after the usual specific staining procedure that links malate oxidation to tetrazolium dye reduction. At least two kinds of supernatant and one kind of mitochondrial MDH in individual fish of each species was demonstrated.

Relative abundance of the various isozymes varies with the tissue of origin. In liver, only one heat stable isozyme is observed in both species, and it is likely constituted mainly of supernatant MDH. In heart tissue four isozymes are observed and the liver supernatant form also predominates. In white muscle the two kinds of supernatant MDH appear to be synthesized in comparable amounts and, including the mitochondrial MDH, four isozymes of nearly equivalent catalytic activity are produced. In walleye the three kinds of MDH homozygotes all showed similar enzymatic properties of their corresponding isozymes.

In contrast to the polymorphic nature of walleye MDH isozymes the closely related sauger are monomorphic for MDH, and a number of analogous isozymes have identical electrophoretic mobility in the two species. More significantly, the major mitochondrial MDH isozymes have different mobility in each species. This fact is suggested as a taxonomic criterion to distinguish the two species. Some very rare fishes, evidently interspecific hybrids, produced three mitochondrial MDH isozymes. It was also possible to hybridize walleye and sauger MDH isozymes in vitro to produce phenotypes indistinguishable from presumed wild hybrid fishes.

CLAYTON, J. W., R. E. K. HARRIS, AND D. N. TRETIAK. 1973. Indentification of supernatant and mitochondrial isozymes of malate dehydrogenase on electropherograms applied to the taxonomic discrimination of walleye (*Stizostedion vitreum vitreum*), sauger (*S. canadense*), and suspected interspecific hybrid fishes. J. Fish. Res. Board Can. 30: 927–938.

Le doré jaune (*Stizostedion vitreum vitreum*) et le doré noir (*S. canadense*) produisent communément quatre isozymes de la malate déshydrogénase (MDH) dans les électrophérogrammes d'extraits de muscle blanc de poissons homozygotes individuels. Nous avons déterminé la stabilité à la chaleur de chaque isozyme, de même que leur réactivité à la nicotinamide-adénine dinucléotide (NAD) et à ses analogues, en mesurant la densité de chaque isozyme sur les électrophérogrammes, après avoir utilisé les techniques de coloration usuelles qui lient l'oxydation du malate à la réduction du colorant tétrazolium. Grâce à cette méthode, on a pu démontrer l'existence d'au moins deux sortes de MDH surnageante et d'une sorte de MDH mitochondrique chez les individus de chaque espèce.

L'abondance relative des divers isozymes varie selon le tissu d'origine. Dans le foie, on n'observe qu'un isozyme stable à la chaleur chez les deuxe spèces, probablement constitué de MDH surnageante. Dans le tissu cardiaque, on observe quatre isozymes, et la forme surnageante du foie prédomine également dans le coeur. Dans le muscle blanc, les deux types de

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MDH surnageante semblent être synthétisés en quantités comparables et produire quatre isozymes, y compris la MDH mitochondrique, à activité catalytique à peu près équivalente. Chez le doré jaune, les trois sortes d'homozygotes pour la MDH possèdent toutes les propriétés enzymatiques de leurs isozymes correspondants.

Contrairement au polymorphisme des isozymes de la MDH du doré jaune, celles du doré noir, son proche parent, sont monomorphiques pour la MDH, et plusieurs isozymes analogues ont une mobilité électrophorétique identique chez les deux espèces. Fait plus significatif, cependant, les principaux isozymes de la MDH mitochondrique ont une mobilité différente chez les deux espèces. Cette particularité peut être un critère taxonomique utile pour distinguer les deux espèces. Quelques rares individus, évidemment des hybrides, produisent trois isozymes de la MDH mitochondrique. Il a été également possible d'hybrider in vitro les isozymes de la MDH du doré jaune avec celles du doré noir, pour produire des phénotypes indiscernables des présumés hybrides sauvages.

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It has recently been shown that walleye (*Stizostedion vitreum vitreum*) have at least three alleles for the most anodal form of supernatant malate dehydrogenase (MDH) (Clayton et al. 1971). It was therefore of interest to investigate the electrophoretic form of MDH in the closely related sauger (*S. canadense*). It soon became apparent that sauger are monomorphic for MDH and, although some of the sauger MDH bands are electrophoretically identical to some walleye bands, there is also a characteristic difference in the MDH phenotypes that readily distinguishes the species.

Evidence from subcellular fractionations in walleye suggested that the species distinctive isozyme was of mitochondrial origin and was the major mitochondrial MDH isozyme in white muscle (Clayton et al. 1971). The subcellular fractionations were somewhat equivocal in that supernatant preparations of cell cytoplasm almost inevitably contain some mitochondrial material and it is also difficult to be certain that mitochondrial preparations are completely free from cytoplasmic enzymes. It is well known that the two forms of MDH, supernatant and mitochondrial, differ in the rate of thermal inactivation (Kitto and Lewis 1967) as well as in reactivity with the coenzyme nicotinamide-adenine dinucleotide (NAD) and coenzyme analogues (Kaplan and Ciotti 1961). In the present work we have applied these techniques in combination with direct measurement of the intensity of isozyme staining on the gel strips to determine the nature of the various isozymes in walleye, sauger, and suspected hybrid fishes.

The hybridization of MDH isozymes in vitro by freezing and thawing muscle extracts in the presence of phosphate has also been demonstrated (Wheat and Whitt 1971). This paper shows how this molecular hybridization technique may be applied to walleye and sauger in combination with the biochemical isozyme classification to support the identification of some rare MDH isozyme phenotypes in wild fishes as phenotypes of interspecific walleye-sauger hybrids.

Materials and Methods

Walleye and sauger were collected from many lakes of the English and Winnipeg River systems in northwestern Ontario by Mr D. W. Brooks, Ontario Department of Lands and Forests. Other fish were collected from the Red River at Lockport, Manitoba, and a few were obtained from various other locations in Manitoba and Saskatchewan.

The coenzymes nicotinamide-adenine dinucleotide (NAD), 3-acetylpyridine-adenine dinucleotide (APAD) and nicotinamide-hypoxanthine dinucleotide (NHD) were obtained from P. L. Biochemicals Inc., Milwaukee. Phenazine methosulfate (PMS), N-(3-aminopropyl)-morpholine and 3-mercapto-1, 2-propanediol were obtained from Aldrich Chemical Company, Milwaukee. Nitro BT [(2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3-3-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride)] was a product of Dajac Laboratories Division of the Borden Chemical Company, Philadelphia. All other chemicals used were of laboratory reagent grade.

Malate dehydrogenase phenotypes of individual wild fish were determined by the starch gel electrophoretic procedure previously described (Clayton et al. 1971).

The response of the isozymes to the coenzyme analogues APAD and NHD was tested by substituting equal amounts of the analogues for NAD in the staining mixture. The heat sensitivity of isozymes was tested by heating individual starch gel strips for varying lengths of time before staining. In this procedure the gel strips were placed individually into plastic trays $4 \times 11 \times 2$ cm. The heating period commenced with the addition, to the gel tray, of 25 ml of buffer solution of the composition used to prepare the starch gel. This buffer was maintained in a temperature-controlled water bath. After the addition of the warm buffer the gel trays were immediately placed in the same water bath. The heating period was ended by quick aspiration of the warm buffer from the tray followed by the addition of ice temperature starch gel buffer. Finally the cold buffer was drawn off and the gel strips were stained in the usual way with NAD as coenzyme. The water bath temperature for each heat denatur-

ation experiment is indicated in the corresponding Figure that gives the results of the experiment. These temperatures vary somewhat and were selected, on the basis of preliminary experiments, to be suitable for the demonstration of a difference in heat stability of the various isozymes. These experiments were conducted with white muscle and heart muscle extracts from C^1C^1, C^2C^2, and C^3C^3 homozygous (supernatant MDH) walleye, sauger, and one presumed interspecific walleye-sauger hybrid. The electrophoresis buffer system for the C^1C^1 walleye was N-(3-aminopropyl)-morpholine-citrate, *p*H 6.1 (Clayton and Tretiak 1972) while for all the other fish the usual tris-(hydroxymethyl)-methylamine (Tris)-citrate system was used.

The gel strips from the coenzyme analogue and thermal inactivation experiments were stained and fixed in methanol-acetic acid-water solution in the usual way. The fixed strips were then sliced to separate the three electrophoretic channels and each portion was transferred individually into a suitable trough-like cuvette (Gilford Instrument Laboratories, Oberlin, Ohio). The gel strip was maintained in the proper position within the cuvette with the aid of a Perspex spacer. The void space in the cuvette was filled with a 1:1 ethanol-water solution. The absorbance of the isozymes, at 525 nm, was measured directly with a Gilford Model 2400 recording spectrophotometer equipped with a linear sample transport device.

In one series of gels the absorbance of each isozyme was measured directly as peak height on the recorder strip chart and also as peak area. The peak area was measured by cutting out and weighing the area under the peak on photocopies of the recorder strip chart.

In the starch gel electrophoresis procedure we use (Tsuyuki et al. 1966) it is not practical to measure the amount of protein extract applied to the sample slot for each channel on a gel and the amounts are variable. To quantitate the absorbance measurements it is necessary to correct for this variable amount of protein. In experiments with muscle extracts this was accomplished by determining the factor necessary to set the absorbance of the BC isozyme in each channel to a constant arbitrary value. The absorbances of the other isozymes in each channel were then corrected by multiplication by the same factor, e.g., $\frac{\text{'set value'}}{\text{observed BC absorbance}}$' for that particular channel. The corrected replicate absorbances (3–6) were then averaged to produce a figure that could be plotted in the graphs for the various heat and coenzyme analogue experiments. The heat stability of the liver MDH of a sauger and a C^1C^2 walleye was investigated similarly except that the tissue extract used for electrophoresis was a mixture of equal parts pig muscle extract and fish liver extract. The mixed extract was necessary because the fish liver electropherograms show no BC isozyme activity that could be used as a 'standard' as in the muscle. In these experiments the major pig muscle supernatant MDH isozyme was used as the 'standard,' as described above, to correct for variable total amounts of enzyme in each electrophoretic channel.

Isozymes in walleye and sauger white muscle extracts were hybridized before electrophoresis by the method of Wheat and Whitt (1971). Electrophoresis was performed in our usual way with Tris-citrate buffers at *p*H 8.0 with extracts that had been subjected to the full hybridization treatment (Wheat and Whitt 1971) as well as with similar muscle extracts that were mixed only 1 hr before electrophoresis. Those extracts given the full treatment were dialysed thoroughly against the extraction buffer prior to electrophoresis.

Results and Discussion

It has already been shown (Clayton et al. 1971) that walleye in western Canada have three alleles for the most anodal form of MDH. The subunits produced by these alleles were designated C^1, C^2, and C^3 in order of increasing electrophoretic mobility. In marked contrast to walleye, sauger from southern Manitoba and northwestern Ontario are monomorphic for MDH and evidently produce only one C subunit with electrophoretic mobility identical to that of the walleye C^3 subunit (Fig. 1). Another common feature of the walleye and sauger electropherograms is the apparent identical mobility of the BB isozyme in the two species. All the sauger were, however, clearly distinguishable from the walleye because of the difference in mobility of the slowest moving isozymes (Fig. 1). It is clear that the walleye are characterized by the presence of a slow-moving intensely staining isozyme (designated AA in Clayton et al. 1971) while sauger electropherograms are devoid of any isozyme in this region. This difference between walleye and sauger is obviously clear and unmistakable and we suggest that it may be used as a definitive taxonomic criterion for distinguishing the species.

In the course of examining many wild fish we observed a few phenotypes that were evidently attributable to interspecific hybrid fishes (Fig. 1) as well as some other rare walleye phenotypes (Fig. 2). The major MDH isozyme phenotypes of the three walleye homozygotes, sauger, and representatives of the unusual rare kinds are all shown diagramatically together with the presumed isozyme composition in Fig. 3. The least anodal common walleye isozyme shown as M^wM^w in Fig. 3 was previously identified as AA (Clayton et al. 1971). In Fig. 2 we identify a rare walleye variant as M^1M^{1S}, with the implication that it is a probable heterozygote for a rare slow electrophoretic form of walleye mitochondrial MDH. In Fig. 3 and through the remainder of the paper this phenotype is identified as M^wM^{ws}. This system of nomenclature was chosen to characterize this rare walleye phenotype as due to a slow (M^{ws}) electrophoretic form of the major (M^w) walleye white muscle mitochondrial MDH. The other unusual walleye, indicated as C^2C^2XX and C^3C^3XX in Fig. 2 and 3 are characterized by

a doublet isozyme in the region of the least anodal walleye M^wM^w isozyme. At the present time the molecular and genetic constitution of this doublet isozyme is unknown to us and we show these phenotypes only for completeness of the record. The presumed major white muscle mitochondrial MDH isozyme in sauger is designated M^sM^s.

The suspected interspecific hybrid phenotypes (Fig. 1) display the three isozymes that would be expected in a hybrid M^wM^w, M^wM^s, and M^sM^s. The most intense staining isozyme is M^wM^s and again this is what would be expected of a hybrid if the isozyme subunits were distributed among the isozymes in the usual binomial way. The frequency at which these suspected hybrid fishes occur is approximately 1% in waters of southern Manitoba and northwestern Ontario (Table 1). All the presumed hybrid fishes found were of either the C^2C^3 or C^3C^3 phenotype (Fig. 1). This may be interpreted as additional circumstantial evidence for the hybrid nature of these fishes because in the walleye populations examined (Table 2) a large fraction of the walleye were C^2C^2 homozygotes. If the presumed hybrid fishes were in fact walleye with some unusual M^wM^w isozymes one would have expected some representation in the C^2C^2 class. If on the other hand they are hybrids then they should have had at least one C^3 gene from the sauger parent and this is exactly what was found. The alternative possibility that the suspected hybrids are sauger with a rare slow electrophoretic variant of mitochondrial MDH is also very unlikely because sauger are monomorphic for the C^3 form of supernatant MDH, and, as may be seen in Table 2, six of the seven alleged hybrids had the C^2C^3 supernatant MDH phenotype that is totally unknown in the several hundred normal sauger that were analyzed (Table 1). The only C^3C^3 suspected hybrid that could possibly be a rare sauger variant (Fig. 1 and Table 2) was found in the Red River. This was also the source of the highest proportion of C^3C^3 walleye (Table 2) encountered in our study and it seems probable that this M^wM^s/C^3C^3 fish was hybrid between sauger and C^3C^3 walleye.

Walleye MDH isozymes were tentatively identified as either mitochondrial or supernatant on the basis of subcellular fractionations (Clayton et al. 1971). These two forms of MDH are known to be distinguishable on the basis of some characteristic physicochemical properties as well as by their subcellular location. It has been the usual practice to isolate and purify the two forms of the enzyme by classical protein chemistry procedures prior to examination of the various physico-chemical properties that distinguish the two forms (Kitto 1966). In the present work we have attempted to evaluate both the relative heat stability of the various isozymes and the response to coenzymes directly on the gel strips. This technique not only eliminates the time consuming and exacting protein-isolation step but it also provides evidence about the electrophoretic isozymes which are sometimes difficult to resolve by other protein-separation techniques.

We have accomplished the physical measurement of enzymatic activity by determining the absorbance of the tetrazolium dye deposited on the gel strip directly with the aid of a recording spectrophotometer. This technique was selected after some preliminary experiments showed that an alternative procedure whereby the electropherograms are first photographed with color transparency film and the developed transparency is scanned in the spectrophotometer (Bailey et al. 1970) did not seem to offer any advantage over scanning the starch gel directly. Other preliminary experiments showed that when gels were stained for varying periods of time the area under the spectrophotometer absorbance curve was directly proportional to the time of staining for all reasonable degrees of isozyme staining intensity. The area under the spectrophotometer curve should be the true measure of catalytic activity. The measurement of the area may be achieved by sophisticated methods (Bailey et al. 1970) or by weighing the paper cut out under the curve. In either case it is time consuming and we reasoned that the spectrophotometer peak height of each isozyme should be proportional to the area because in the gel electrophoresis technique we use (Tsuyuki et al. 1966) there is little diffusion of protein zones and all isozymes are of very similar width. A comparison of peak area and peak height showed a very good correlation ($r = 0.96$) and we have therefore used peak height as a measure of catalytic activity. The only exceptions occurred when it was obvious that the spectrophotometer could not resolve an isozyme doublet and are clearly indicated where they occur.

White muscle MDH isozymes in both walleye and sauger are clearly distinguished by their susceptibility to heat denaturation (Fig. 4). In the sauger extract M^sM^s and BB isozymes appear as a doublet (Fig. 1, 2, 3, and 1O) and the spectrophotometer did not resolve them (Fig. 4a). It was therefore necessary to evaluate these isozymes together by the peak area procedure and in this particular experiment the BC^3 isozyme was also measured by area. The results clearly show that relative to the BC^3 isozyme the combination of M^sM^s + BB shows a marked loss in catalytic activity during the early part of the heating period but then the activity levels off. We interpret this as typical of the behavior of a combination of a heat sensitive M^sM^s mito-

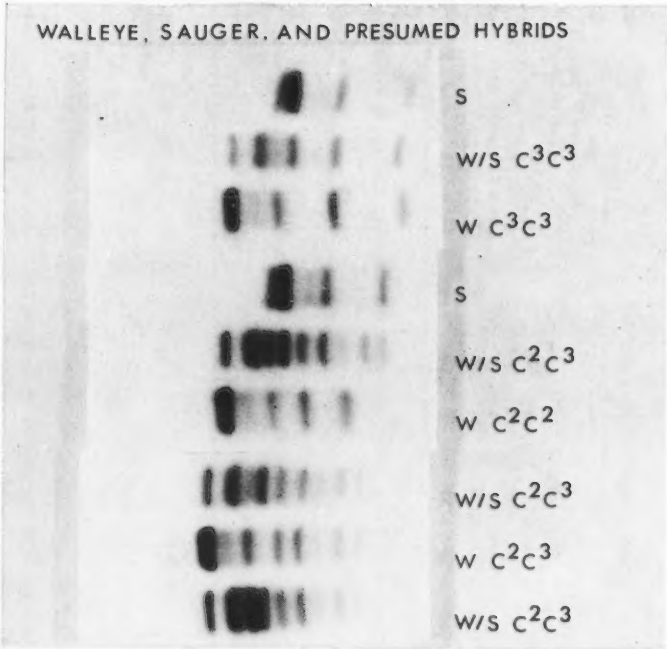

FIG. 1. Electropherograms of MDH isozymes in walleye, sauger, and presumed interspecific hybrids. S = sauger; W/S = walleye-sauger presumed hybrid; W = walleye.

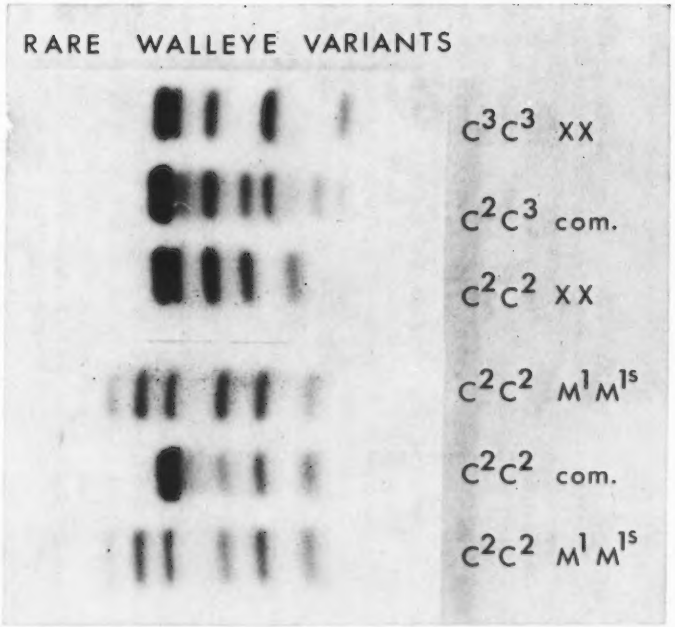

FIG. 2. Electropherograms of normal and rare walleye MDH phenotypes. COM. = one of the six common walleye MDH phenotypes; XX = walleye with unusual doublet of slow isozymes; M^1M^{1S} = walleye evidently heterozygous for a slow variant (M^{1S}) of the assumed major mitochondrial (M^1) isozyme.

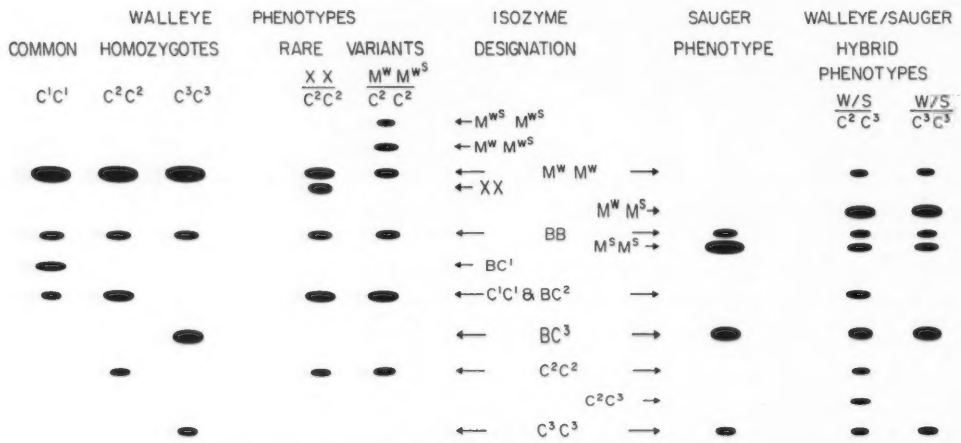

FIG. 3. Diagram of walleye, sauger and interspecific hybrid MDH isozyme phenotypes showing presumed subunit composition of the isozymes.

chondrial MDH isozyme and a heat resistant BB supernatant MDH isozyme. The electrophoresis at *p*H 6.1 (C^1C^1 walleye, Fig. 4b) resolves an additional MDH isozyme (M^zM^z) that is presumed to be of the mitochondrial type. The *p*H 6.1 electrophoresis was required to produce an adequate separation of the BB, BC^1, and C^1C^1 isozymes (Clayton and Tretiak 1972). As is shown in Fig. 4b both these presumed mitochondrial isozymes were heat labile. The resolution of an additional mitochondrial isozyme also occurred when C^2C^2 walleye MDH electropherograms were prepared with *p*H 6.5 electrophoresis buffers (Clayton et al. 1971). The MDH isozymes of C^2C^2 and C^3C^3 walleye (Fig. 4c and d) were also clearly distinguished on the basis of heat stability. In the C^3C^3 walleye (Fig. 4d) the C^3C^3 isozyme was also resistant to heat denaturation. The CC isozymes in electropherograms from the other fish were very weakly stained and were not shown in Fig. 4. It seems clear from Fig. 4 that the MDH isozymes in white muscle are readily distinguished on the basis of resistance to heat denaturation and that the least anodal major isozyme in walleye (M^wM^w) and sauger (M^sM^s) is a heat labile mitochondrial MDH isozyme. Our earlier subcellular fractionation experiments (Clayton et al. 1971) indicated that the BB isozyme might be made up of both supernatant and mitochondrial MDH isozymes of coincident electrophoretic mobility. The data of Fig. 4 do not suggest the presence of any significant amount of mitochondrial MDH (heat labile) enzymatic activity in the BB isozyme.

The response of the white muscle isozymes to coenzyme analogues (Fig. 5) evidently parallels the heat denaturation results. Thus in the walleye the presumed mitochondrial MDH isozymes M^wM^w and M^zM^z both show a marked enhancement in apparent activity when reacted with the coenzyme analogue APAD in comparison to NAD or NHD. It has been shown that susceptibility to heat denaturation and high reactivity with APAD are characteristic of vertebrate mitochondrial MDH (Kitto 1966) and it seems clear that these slow electrophoretic isozymes are in fact mitochondrial MDH isozymes.

TABLE 1. Occurrence of suspected walleye/sauger hybrids in waters of southern Manitoba and northwestern Ontario.

Locality	Number of fish analyzed			Rare walleye MDH
	Walleye	Sauger	Hybrids	
Lake Winnipeg, Man.	86	121	0	0
Red River at Lockport, Man.	47	150	3	0
English River system northwest Ontario	230	22	2	0
Lake of the Woods northwest Ontario	292	178	2	4

TABLE 2. Comparison of supernatant malate dehydrogenase phenotypes of presumed hybrids and in walleye populations.[a]

Location	Walleye			Presumed hybrids			Rare walleye mitochondrial MDH		
	C^2C^2	C^2C^3	C^3C^3	C^2C^2	C^2C^3	C^3C^3	C^2C^2	C^2C^3	C^3C^3
Red River at Lockport, Man.	16	20	10	–	2	1	–	–	–
English River system northwest Ontario	119	69	17	–	2	–	–	–	–
Lake of the Woods northwest Ontario	116	118	40	–	2	–	3	–	1

[a]Walleye with C^1 phenotypes are rare in these populations and although some were observed they were left out of this table.

In the sauger (Fig. 5a) it was necessary to evaluate both the M^sM^s and the BB isozymes together. Despite this problem the heat lability (Fig. 4a) and the differential reactivity with coenzyme analogues clearly show that the major isozyme of this doublet is of the mitochondrial type. The activities of the CC isozymes were quite low and the differences in reactivity shown in Fig. 5 are probably insignificant except possibly for the sauger. In this example the modest increase in reactivity in the series APAD, NAD, NHD is unexpected. Previous studies with tuna supernatant MDH and coenzymes showed the rate of reaction to decline in the order APAD>NAD>>NHD. A similar study with and spring salmon, another species like walleye sauger with two kinds of supernatant MDH, (Bailey et al. 1970) showed the activity with APAD for both supernatant enzymes to be 1.4 times the activity with NAD but no tests were made with NHD.

Although it was not possible to examine the sauger BB isozyme separately, the three walleye BB isozymes were resolved well by electrophoresis and all

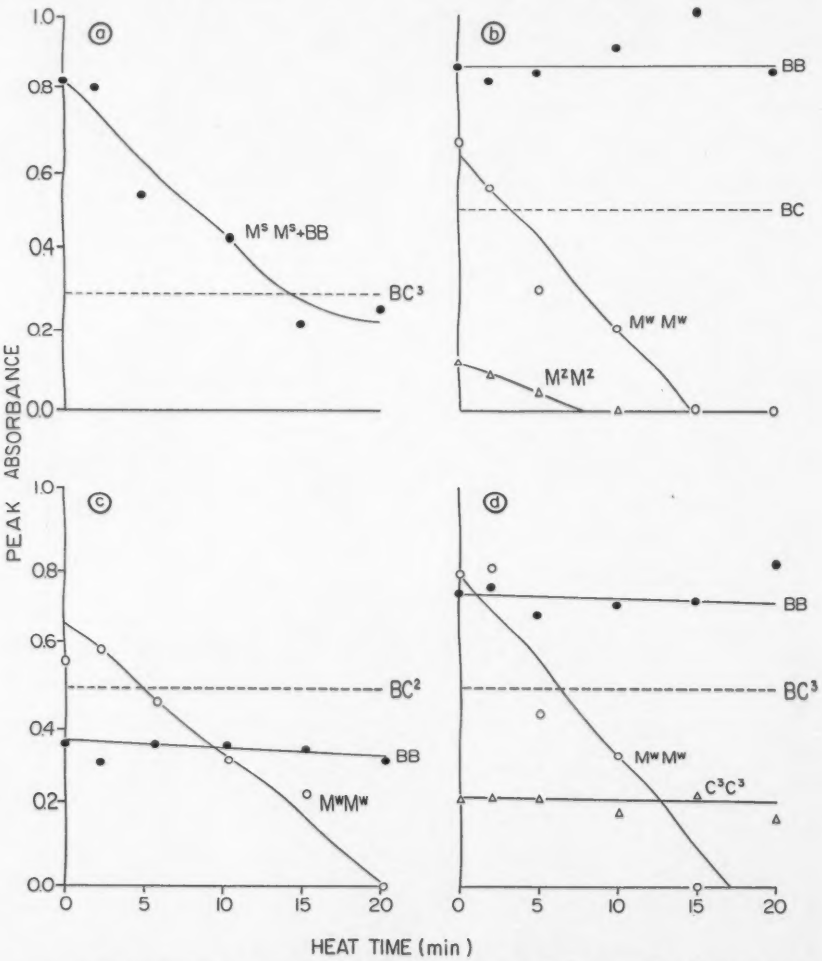

FIG. 4. Heat denaturation of white muscle MDH isozymes on starch gel strips: (a) Sauger heated at 42.5 C; (b) C^1C^1 walleye heated at 37 C; (c) C^2C^2 walleye heated at 40 C; (d) C^3C^3 walleye heated at 42.5 C.

three show a slight diminution of activity for the coenzyme series APAD > NAD >NHD. When these data are considered together with the heat inactivation data of Fig. 4 it seems clear that the heat labile M^wM^w isozyme, with high reactivity with APAD relative to NAD and NHD, is of mitochondrial origin. Also, there is little doubt that the heat stable CC isozyme, that shows little difference in reactivity between the coenzyme analogues, is an authentic supernatant MDH isozyme. The data for the BB isozyme are less conclusive, and although it is clear that most of the catalytic activity here is of the supernatant type on the basis of considerable heat stability (Fig. 4), the slight increase in activity with the APAD coenzyme analogue could be interpreted as a reflection of a small amount of a mitochondrial form of the enzyme with electrophoretic mobility coincident with the major supernatant component as was suggested earlier (Clayton et al. 1971) on the basis of subcellular fractionation studies.

The responses of MDH isozymes in heart muscle to heat treatment and reaction with coenzyme analogues (Fig. 6 and 7) were quite similar to the corresponding studies with white muscle isozymes. On the other hand the total relative enzymatic activities of the isozymes are quite different, and while in white muscle the BB and BC isozymes (Fig. 4 and 5) are approximately equivalent in catalytic activity as reflected by isozyme absorbance, in heart tissue the absorbances of the BB isozymes exceeded the BC absorbances by at least threefold (Fig. 6 and 7). The response of the C^3C^3 walleye M^wM^w and BB isozymes to APAD (Fig. 7d) was

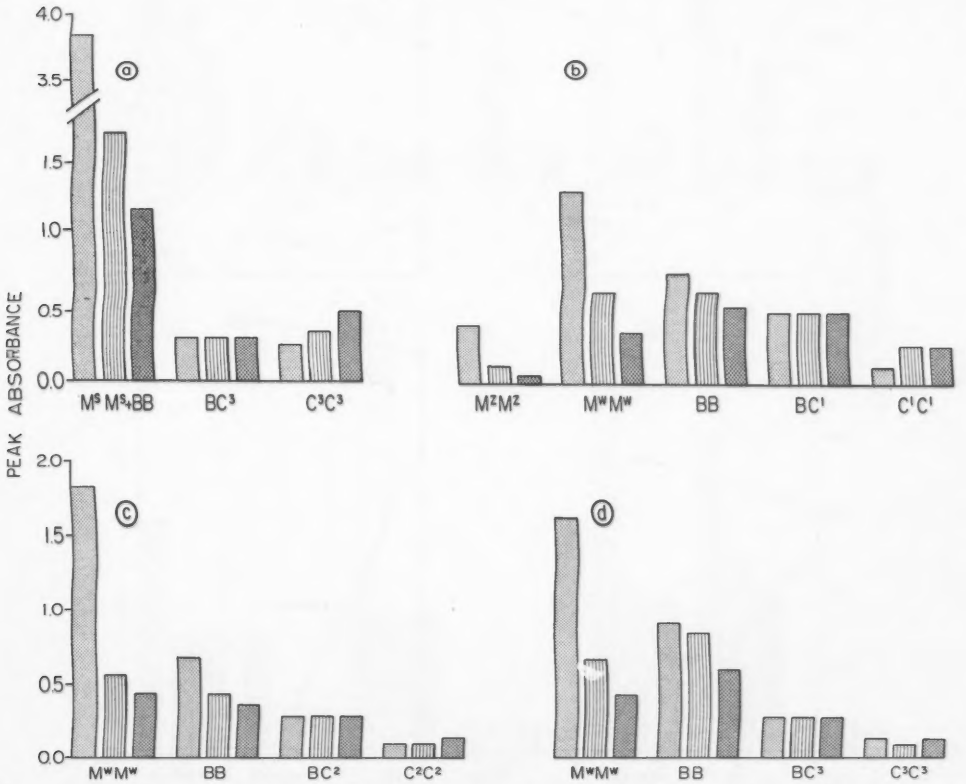

FIG. 5. Catalytic activity of walleye and sauger white muscle MDH isozymes with the natural coenzyme nicotinamide–adenine dinucleotide (NAD) and analogues. (a) Sauger; (b) C^1C^1 walleye; (c) C^2C^2 walleye; (d) C^3C^3 walleye. (light stipple = acetylpyridine–adenine dinucleotide (APAD); vertical hatching = NAD; dark stipple = nicotinamide–hypoxanthine dinucleotide (NHD).)

greater than that observed in any other sample. Also the activity of the M^wM^w isozyme in C^3C^3 walleye was the most heat labile (Fig. 6d). We interpret these results as further evidence in these fish for the well known, but poorly understood, lability of mitochondrial MDH (Bailey et al. 1970; Wheat et al. 1971). The homogenate of C^3C^3 heart tissue utilized in these experiments probably contained a fortuitously high level of mitochondrial MDH activity that was partially destroyed before electrophoresis in the other extracts. The apparent increase in activity of the BB isozymes in all fish (Fig. 6) during the heating period is of course unreal and is an artifact produced by the technique of comparison of all activities in a given channel on each gel to the activity of the BC isozyme. Thus the apparent increase in activity of the BB isozyme with time of heating shows only that the BB isozyme is more heat stable than the BC isozyme that was arbitrarily chosen as the standard for comparison. The sauger heart extract produced an additional heat sensitive isozyme, M^TM^T (Fig 6 and 7) not seen in white

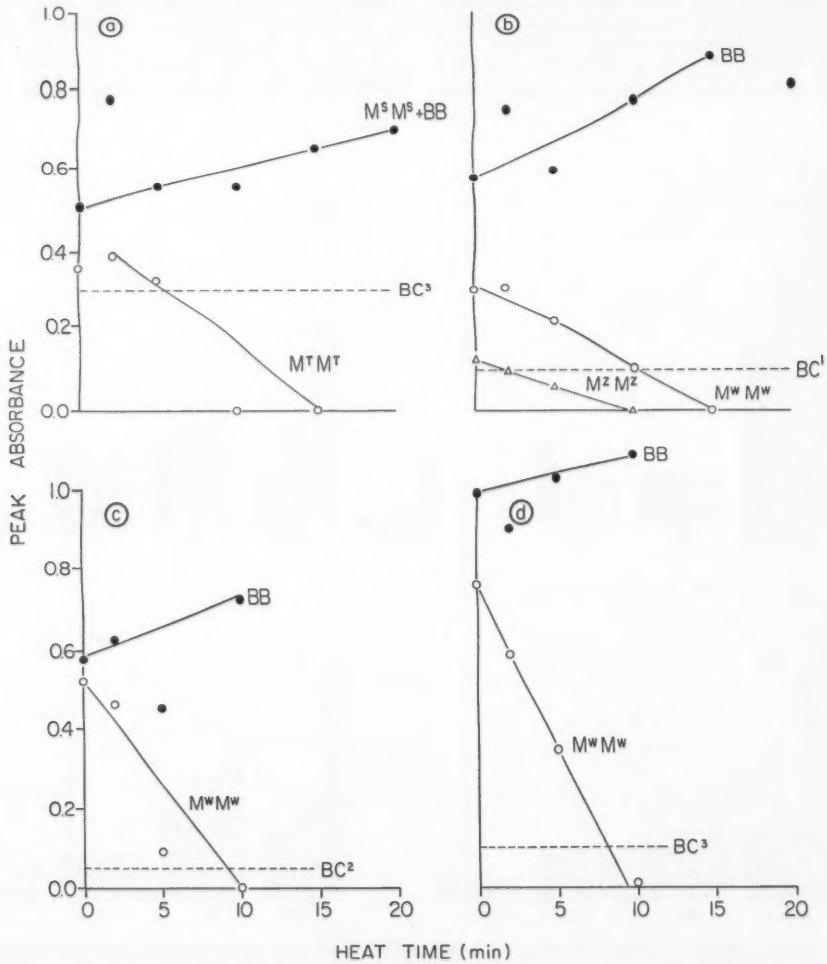

FIG. 6. Heat denaturation of heart muscle MDH isozymes on starch gel strips. (a) Sauger; (b) C^1C^1 walleye; (c) C^2C^2 walleye; (d) C^3C^3 walleye. C^1C^1 walleye heated at 37 C; all others 42.5 C.

ouscle electropherograms. As in white muscle the mrmal sauger mitochondrial isozyme (M^sM^s) and the BB isozyme were not well resolved and were estimated together by the peak area procedure. The overwhelming abundance of heat stable enzymatic activity in the BB electrophoretic zone is indicated by the modest apparent increase in activity of the BB isozyme during the heating period in each experiment. Electrophoresis at *p*H 6.1 revealed and additional isozyme in the C^1C^1 heart extract (M^zM^z, Fig. 6b and 7b) which corresponded in heat lability and electrophoretic mobility with what we presume was the same isozyme produced under similar conditions from a white muscle extract (Fig. 4b and 5b).

Extracts from liver tissue of both walleye and sauger produced a single MDH isozyme. The electrophoretic mobility of this isozyme appeared to be the same in both species; furthermore, the mobility of the liver MDH isozyme and the muscle BB isozyme were apparently identical. It has already been shown that the BB isozyme in muscle tissue

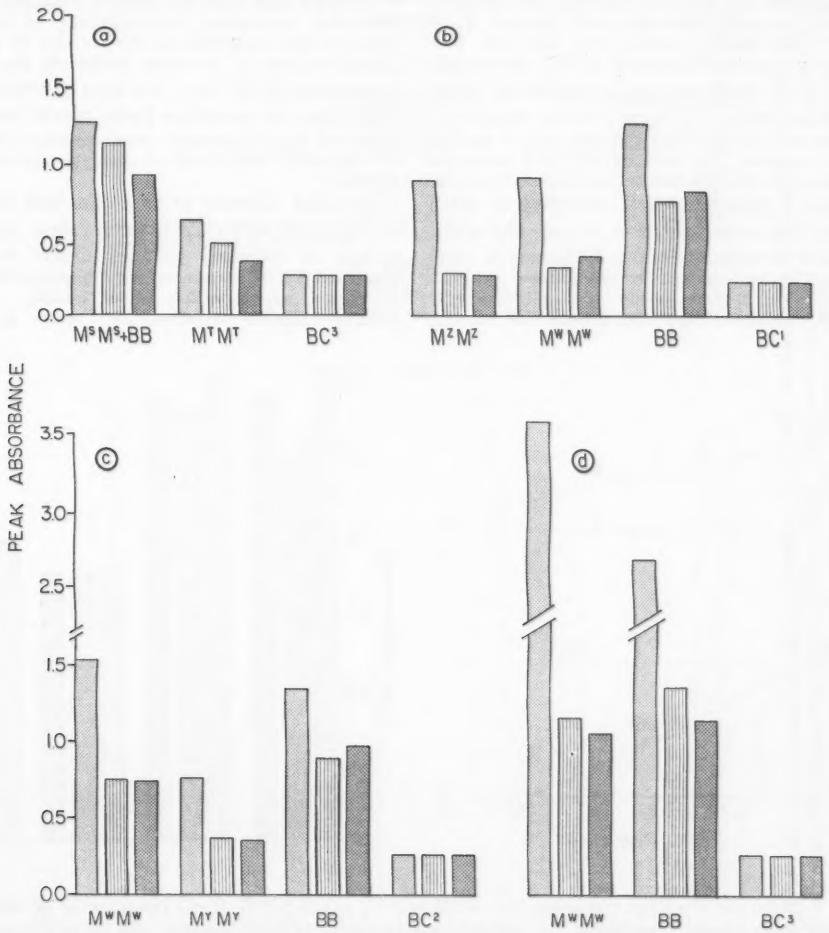

FIG. 7. Catalytic activity of walleye and sauger heart muscle MDH isozymes with the natural coenzyme nicotinamide-adenine dinucleotide (NAD) and analogues. (a) Sauger; (b) C^1C^1 walleye; (c) C^2C^2 walleye; (d) C^3C^3 walleye. (light stipple = acetylpyridine-adenine dinucleotide (APAD); vertical hatching = NAD; dark stipple = nicotinamide-hypoxanthine dinucleotide (NHD).)

has properties normally associated with supernatant MDH. On the other hand it was previously shown (Clayton et al. 1971) that a preparation of walleye liver mitochondria yielded an MDH isozyme with the same mobility as that found in whole liver tissue extract. It therefore seemed important to investigate the nature of the liver MDH in walleye and sauger with regard to heat stability and response to coenzyme analogues.

The presence of only one detectable MDH isozyme in walleye and sauger liver presented a problem since there was no other isozyme to use as a standard to correct for variable total amounts of protein in each electrophoresis channel. Preliminary experiments showed that the fish liver isozyme was electrophoretically readily distinguishable from the MDH isozymes in pig muscle tissue. Also, there seemed to be no obvious interaction between the fish liver isozyme and any of the pig muscle isozymes. The heat stability and coenzyme analogue (Fig. 8) experiments were therefore carried out with a protein solution composed of equal parts pig muscle and fish liver extract. The major pig muscle supernatant isozyme (Chilson et al. 1966) was used as the standard for comparison. As may be seen in Fig. 8, there seems to be no difference in the heat stability of the sauger liver MDH isozyme and the pig muscle supernatant MDH. The walleye liver MDH was also quite heat stable and it is clear that both fish liver isozymes were much more resistant to heat denaturation than the pig muscle mitochondrial isozyme. In Fig. 8 the open and closed symbols for pig mitochondrial MDH were obtained from the experiment with walleye and sauger liver MDH, respectively.

The response of the fish liver isozymes (Fig. 8) to NAD and the analogues APAD and NHD is rather unusual in that both analogues appear to enhance the activity of the enzyme. Again it is well to consider that these are relative data and in this particular exepriment the activities of the fish enzymes are compared to the activity of the pig muscle enzyme. If, as seems likely, the pig muscle supernatant MDH had a low level of activity with NHD, then the correction factor would have been large and the fish enzyme may appear to have an unrealistically high level of catalytic activity with NHD.

The main inference to be drawn from the data of Fig. 8 is still clear however; these fish liver extracts as ordinarily prepared display little evidence of MDH isozymes with appreciable levels of typical mitochondrial MDH activity. An apparently similar situation also exists in saury

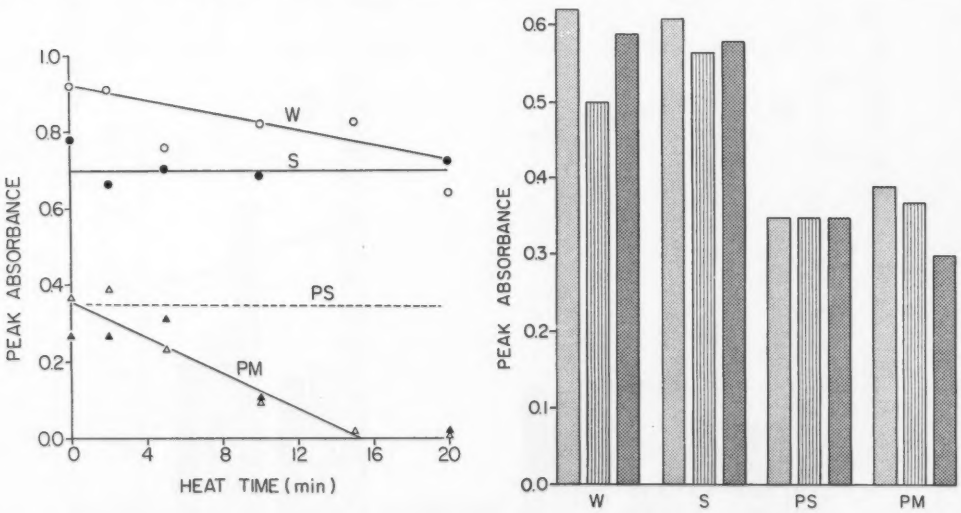

FIG. 8. Comparison of walleye and sauger liver MDH with pig muscle MDH. (*Left*) Denaturation of MDH isozymes on starch gel strips by heating at 55 C. (*Right*) Catalytic activity of MDH isozymes with the natural coenzyme nicotinamide-adenine dinucleotide (NAD) and analogues. (W = walleye liver MDH; S = sauger liver MDH; PS = major pig muscle supernatant MDH isozyme; PM = major pig muscle mitochondrial MDH isozyme; light stipple = acetylpyridine-adenine dinucleotide (APAD); vertical hatching = NAD; dark stipple = nicotinamide-hypoxanthine dinucleotide (NHD).)

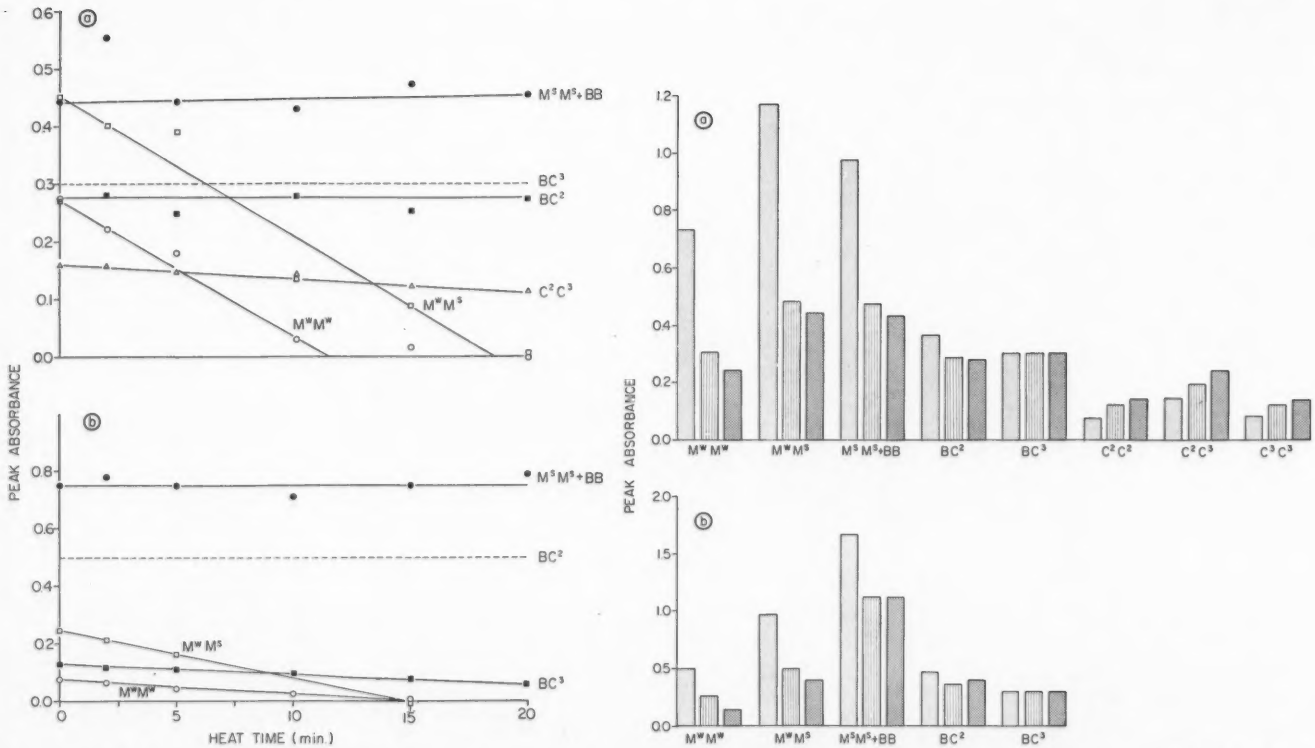

FIG. 9. (*Left*) Heat denaturation of MDH isozymes in a presumed walleye–sauger interspecific hybrid (a) white muscle MDH isozymes heated at 42.5 C; (b) heart muscle MDH isozymes heated at 40 C. (*Right*) Catalytic activity of MDH isozymes, in a presumed walleye–sauger interspecific hybrid, with nicotinamide-adenine dinucleotide (NAD) and analogues. (a) white muscle MDH isozymes; (b) heart muscle MDH isozymes. (light stipple = acetylpyridine–adenine dinucleotide (APAD); vertical hatching = NAD; dark stipple = nicotinamide-hypoxanthine dinucleotide (NHD).)

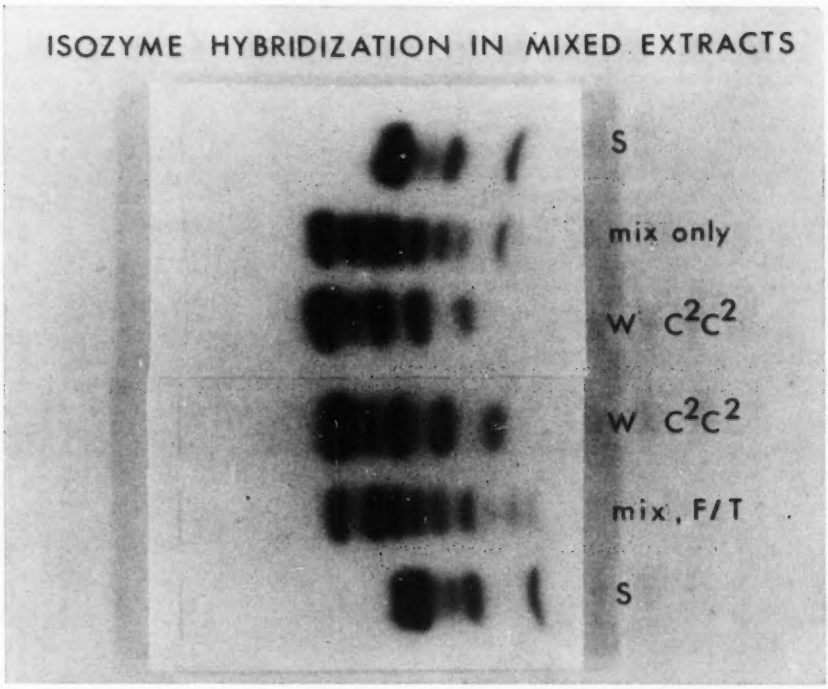

FIG. 10. Electropherograms of MDH isozymes in white muscle extracts from walleye and sauger. (S = sauger; W C^2C^2 = typical C^2C^2 walleye; mix only = a simple mixture of walleye and sauger extracts; mix, F/T = a mixture of walleye and sauger extracts frozen and thawed as described in Methods.)

(*Cololabis saira*) where only one MDH isozyme was found in liver with slow electrophoretic mobility evidently entirely analogous to what we designate as the BB isozyme (Numachi 1970). The data of Wheat et al. (1971) regarding MDH isozymes in bass (*Micropterus*) species and hybrids also reveal little evidence for mitochondrial MDH activity in electropherograms of liver extracts. The possibility exists that the fish livers do contain a mitochondrial MDH with properties not seen in other vertebrate MDH enzymes. Apparently only the mitochondrial MDH in two *Drosophila* species has been shown to be more resistant to heat denaturation than the corresponding supernatant enzyme (McReynolds and Kitto 1970). On the other hand, even this insect mitochondrial MDH retained the characteristic high relative level of reactivity with the APAD coenzyme analogue, while the fish liver enzyme does not. Another possible explanation for our results is that the mitochondrial MDH in the fish livers is in some way inactivated in our procedures and does not survive until the gel strips are stained. Thiols are frequently used as mitochondrial enzyme protective agents (Bailey et al. 1969) but when 3–mercapto–1,2–propanediol was included in all solutions used to prepare walleye and sauger liver MDH electropherograms no additional isozymes were seen. Taken in total the present evidence suggests that synthesis of the typical muscle mitochondrial MDH and CC supernatant MDH in walleye or sauger liver is very low indeed relative to the BB isozyme. Also there is really nothing to suggest that any significant amount of mitochondrial MDH is present in the fish liver BB isozymes.

Our analysis of the nature of the electropherograms of some wild fishes (Fig. 1–3) as interspecific walleye-sauger hybrids with a unique hybrid mitochondrial MDH isozyme composed of both a walleye and a sauger mitochondrial MDH subunit is supported by the heat denaturation and coenzyme analogue studies of the MDH isozymes in a presumed C^2C^3 walleye-sauger hybrid (Fig. 9). The presumed M^wM^s hybrid isozyme is readily heat denatured (Fig. 9) while the BB and BC isozymes are little affected. Similarly in Fig. 9 this isozyme displays a considerable enhancement in activity with the APAD coenzyme analogue.

The heart tissue extracts from the suspected hybrid fish (Fig. 9b) show the same asymmetric synthesis favoring B subunits that was observed in the pure species (Fig. 6 and 7). The resolution of the heart tissue isozymes was normal in the coenzyme analogue experiment in spite of the magnitude of the M^sM^s + BB isozyme and the relative activities of the M^sM^s + BB and BC isozymes (Fig. 9b) appeared to be similar to the other experiments. In the heat denaturation experiment the resolution of the isozymes was not as good and the BC^2 isozyme appears to have considerably more activity (Fig. 9b) than might have been expected. These minor problems do not detract from the main conclusion to be drawn from the data in Fig. 9 that the presumed hybrid fish produced clearly resolved M^wM^w and M^wM^s MDH isozymes that had the properties expected of a walleye and a walleye-sauger hybrid mitochondrial MDH isozyme. The M^sM^s + BB isozyme in sauger white muscle showed a marked sensitivity to heat inactivation (Fig. 4) while in the suspected hybrid fish the activity of this isozyme (Fig. 9) was little affected by heating. The latter result is apparently due to the fact that most of the M^s subunits appear in the M^wM^s isozyme in the presumed hybrid and the catalytic activity of the M^sM^s + BB isozyme is largely due to the BB component.

It has recently been shown that largemouth and smallmouth bass species have distinctive MDH isozymes that may be hybridized by freezing and thawing a mixed protein extract (Wheat and Whitt 1971). In the bass the distinctive isozymes were supernatant MDH and there was no hybridization when tissue extracts were only mixed. We have conducted similar experiments with walleye and sauger extracts (Fig. 10). It may be seen that the mitochondrial MDH in walleye and sauger hybridizes to some extent when the tissue extracts are mixed. The distribution of the enzymatic activity between the M^wM^w, M^wM^s, and M^sM^s isozyme only approached the expected 1 : 2 : 1 ratio when we used the full freeze-thaw treatment of Wheat and Whitt (1971). Also, the supernatant walleye and sauger isozymes, like the bass isozymes, were only hybridized with the full treatment. The virtual identity of the isozyme pattern produced by freezing and thawing (Fig. 10) with that of presumed wild hybrid fishes (Fig. 1 and 2) offers further support for our identification of these rare fishes as interspecific hybrids.

Acknowledgments

Mr R. M. Hanson of the Freshwater Institute collected fish from the Red River that were used in this study. Mr G. Gray was helpful in providing other specimens that were collected in connection with other Freshwater Institute research programs.

BAILEY, G. S., G. T. COCKS, AND A. C. WILSON. 1969. Gene duplication in fishes: Malate dehydrogenases of salmon and trout. Biochem. Biophys. Res. Comm. 34: 605–612.

BAILEY, G. S., A. C. WILSON, J. E. HALVER, AND C. L. JOHNSON. 1970. Multiple forms of supernatant malate dehydrogenase in salmonid fishes. Biochem-

ical, immunological and genetic studies. J. Biol. Chem. 245: 5927–5940.

CHILSON, O. P., G. B. KITTO, J. PUDLES, AND N. O. KAPLAN. 1966. Reversible inactivation of dehydrogenases. J. Biol. Chem. 241: 2431–2445.

CLAYTON, J. W., AND D. N. TRETIAK. 1972. Amine-citrate buffers for *p*H control in starch gel electrophoresis. J. Fish. Res. Board Can. 29: 1169–1172.

CLAYTON, J. W., D. N. TRETIAK, AND A. H. KOOYMAN. 1971. Genetics of multiple malate dehydrogenase isozymes in skeletal muscle of walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. 28: 1005–1008.

KAPLAN, N. O., AND M. M. CIOTTI. 1961. Evolution and differentiation of dehydrogenases. Ann. N. Y. Acad. Sci. 99: 701–720.

KITTO, G. B. 1966. The comparative enzymology of malate dehydrogenases. Ph.D. Thesis. Brandeis Univ., Waltham, Mass. 381 p.

KITTO, G. B., AND R. G. LEWIS. 1967. Purification and properties of tuna supernatant and mitochondrial malate dehydrogenase. Biochim. Biophys. Acta 139: 1–15.

MCREYNOLDS, M. S., AND G. B. KITTO. 1970. Purification and properties of drosophila malate dehydrogenases. Biochim. Biophys. Acta 198: 165–175.

NUMACHI, K. 1970. Polymorphism of malate dehydrogenase and genetic structure of juvenile population in saury (*Cololabis saira*). Bull. Jap. Soc. Sci. Fish. 36: 1235–1241.

TSUYUKI, H., E. ROBERTS, R. H. KERR, AND A. P. RONALD. 1966. Micro starch gel electrophoresis. J. Fish. Res. Board Can. 23: 929–933.

WHEAT, T. E., W. F. CHILDERS, E. T. MILLER, AND G. S. WHITT. 1971. Genetic and *in vitro* molecular hybridization of malate dehydrogenase isozymes in interspecific bass (*Micropterus*) hybrids. Anim. Blood Grps. Biochem. Genet. 2: 3–14.

WHEAT, T. E., AND G. S. WHITT. 1971. *In vivo* and *in vitro* molecular hybridization of malate dehydrogenase isozymes. Experientia 27: 647–648.

Delay-Differential Equation Models for Fisheries

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WALTER, G. G. 1973. Delay-differential equation models for fisheries. J. Fish. Res. Board Can. 30: 939–945.

Two new "simple" fishery models based on delay-differential equations are introduced and compared to three currently used differential equation models. These new models can account for reproductive lag and allow oscillatory behavior of population biomass, but require only catch and effort data for their application. Equilibrium levels are calculated for both models and examples of various types of growth curves are given. Levels of fishing effort which maximize yield are calculated and found in one case to depend on the previous population and in the other to be constant.

WALTER, G. G. 1973. Delay-differential equation models for fisheries. J. Fish. Res. Board Can. 30: 939–945

Nous présentons deux modèles "simples" de pêcheries, basés sur des équations délai-différentielles, que nous comparons à trois modèles d'équations différentielles d'usage courant. Ces nouveaux modèles tiennent compte d'un décalage de la reproduction et d'un comportement oscillatoire de la biomasse de la population, mais ne requièrent, pour leur application, que des données sur les prises et l'effort de pêche. Nous calculons les niveaux d'équilibre pour les deux modèles et donnons des exemples de divers types de courbes de croissance. Nous calculons les niveaux d'effort de pêche qui donnent un rendement maximal et constatons que dans un cas ils dépendent de la population antérieure, et que dans l'autre ils sont constants.

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Two types of mathematical models have been used in fisheries management. One type exemplified by the detailed models of Beverton and Holt (1957) or Ricker (1958) is applicable to fisheries which have been intensively studied and for which a great number of data regarding mortality and growth rate are available. The other type consists of the simple models proposed by Schaefer (1954), Pella and Tomlinson (1969), and Fox (1970) which use fewer data, but have a number of fundamental shortcomings. However, this second type has rather more widespread applications to fisheries which have not been intensively studied but for which, nonetheless, a need for regulation exists.

In a recent paper, Silliman (1971) pointed out some of the shortcomings of these simple models. Chief among these is the assumption that the time lag between spawning and recruitment has a negligible effect of population growth. Although this is true for some species, in others it is clearly not the case. This study deals with yet a different simple model which overcomes this shortcoming but still requires only catch and effort data. Its parameters are determined in much the same way as in the Schaefer and Fox models, and the optimum level of fishing intensity can be determined in a similar manner. A variant of this model has been applied by Walter and Hogman (1971) to the fishery of northern Green Bay.

Three instantaneous models — The three simple models of Schaefer, Pella and Tomlinson, and Fox consist of differential equations which, in the absence of fishing, assume the form

$$\text{(i)}\quad \frac{1}{P}\frac{dP}{dt} = b - aP$$

$$\text{(ii)}\quad \frac{1}{P}\frac{dP}{dt} = b - aP^{M-1} \qquad (1)$$

$$\text{(iii)}\quad \frac{1}{P}\frac{dP}{dt} = b - a \log P$$

where P is the biomass of "catchable" fish in the fishery and a, b, and M are parameters determined from catch data. Equations (i) and (iii) are special cases of (ii) corresponding to $M = 2$, and $M \to 1$,

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respectively. The growth curves of these equations are given by the following functions:

$$\text{(i)}\quad P(t) = \left\{\frac{a}{b}(1 - e^{-bt}) + P_0^{-1} e^{-bt}\right\}^{-1}$$

$$\text{(ii)}\quad P(t) = \left\{\frac{a}{b}(1 - e^{b(1-M)t}) + P_0^{1-M} e^{b(1-M)t}\right\}^{\frac{1}{1-M}} \quad (2)$$

$$\text{(iii)}\quad P(t) = \exp\left(\frac{b}{a}(1 - e^{-at}) + e^{-at} \log P_0\right).$$

Each of these growth curves behaves in approximately the same way although they have different inflection points (see Fig. 1). The population biomass starts from a certain level and gradually approaches an upper limit with the rate of growth slowing down as the limit is approached. This limit is also the equilibrium level at which the population biomass neither increases nor decreases. This equilibrium level P_∞ may be calculated by setting the left side of each equation in (1) equal to zero:

$$\text{(i)}\quad P_\infty = b/a$$

$$\text{(ii)}\quad P_\infty = (b/a)^{1/(M-1)} \quad (3)$$

$$\text{(iii)}\quad P_\infty = e^{(b/a)}.$$

This points up another shortcoming of these models, namely that the population is always increasing although the rate of increase approaches zero as $P(t)$ approaches P_∞. If an exotic species were introduced into a strange environment these models all suggest that its population biomass would increase gradually until equilibrium was reached. This isn't the sort of behavior one would always expect. One might often expect the population to grow until it exceeded the equilibrium level, then decline again. Indeed this appears to have happened with Lake Michigan alewives. The monotonic nature of these growth curves leads to even stranger behavior if the population happens to exceed the equilibrium level. Each of these models predicts a gradual decline to P_∞ from above, i.e. a gradual decline to the maximum population the environment can sustain. This clearly contradicts our intuition. Instead, once a population has exceeded this level we would expect its mortality rate to increase rapidly until its population were well below that level whereupon it would increase again. In other words, what we might expect is an oscillatory sort of behavior centered about the equilibrium level. Such behavior occurs in none of the three models considered above. We shall consider a pair of models in the form of delay-differential equations which meet this objection as well.

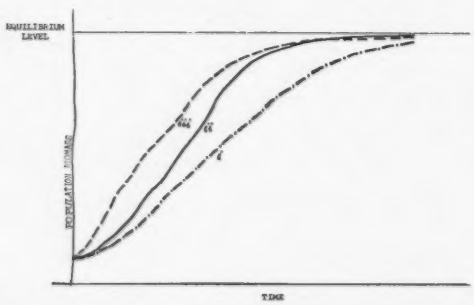

FIG. 1. Growth curves for the instantaneous models of Schaefer ($a = 1$, $b = 100$) (i), Pella and Tomlinson ($a = 1$, $b = 10{,}000$, $M = 3$) (ii), and Fox ($a = 1$, $b = 4.6$) (iii). Initial population is taken as 10% of the equilibrium level $P_\infty = 100$ in each case.

Before we go into these new models, we consider some of the other aspects of the three models mentioned previously. When fishing enters the picture it causes an increase in the mortality rate which is usually assumed proportional to the fishing effort. This is reflected in each of the equations by subtracting a term qf from the right side, i.e. the equations all assume the form

$$\frac{1}{P}\frac{dP}{dt} = G(P) - qf \quad (4)$$

where $G(P)$ is any of the functions on the right side of (1). Here f is the fishing effort and q the "coefficient of catchability." The equilibrium curve along which fishing mortality balances natural growth *then is given by the formula*

$$G(P) - qf = 0. \quad (5)$$

This formula can then be used to find the formula for the equilibrium yield curve since yield y is given by $y = qPf$. It is

$$y = PG(P). \quad (6)$$

To find the level of equilibrium population which maximizes yield, $\frac{dy}{dP}$ is set equal to zero and the equation solved for P. Equation (5) is then used to determine the level of fishing effort which maximizes equilibrium yield.

In each of those equations the parameters may be estimated by fitting the best equation of the form

$$G(P) - qf - \frac{1}{P}\frac{\Delta P}{\Delta t} = 0$$

to catch and effort data, although other approaches give better estimates (see Pella and Tomlinson (1969)).

Two time-lag models — The equations for the new models have the same form as the ones mentioned previously except that a term involving the population a number of years previously is included. They assume that the rate of growth of fish population biomass has two components, one due to instantaneous growth and mortality and the other due to recruitment, and/or lags in density dependent growth and mortality. They further assume that the latter component is proportional to the biomass of the population at some previous time either directly or inversely since no assumptions are made about its sign.

Although the equations have a more complex form, their application to the problem of fishery regulation is no more difficult than the ones mentioned previously.

Two simple time-lag equations are considered, the first of which generalizes Schaefer's equation and the second of which generalizes Fox's. The basic equations are

$$\text{(a)}\ \frac{1}{P}\frac{dP}{dt} = b - a_1 P(t) + a_2 P(t-\omega)$$
$$\text{(b)}\ \frac{1}{P}\frac{dP}{dt} = b - a_1 \log P(t) + a_2 \log P(t-\omega). \tag{7}$$

Here ω is a unit of time representing the lag between spawning and recruitment, or in effect of the population density. The solution to these equations is not well determined by the population at some instant of time. Rather it is necessary to specify the *population over a period of time of length* ω *in* order to get a unique solution.

Equation (7a) is similar to the time-lag equation studied by Wangersky and Cunningham (1957) except that they take $a_1 = 0$. Various possible solution curves of such equations are given and indicate that even their simpler version meets many of the objections given above.

An explicit solution may be obtained if the population is at a constant level P_0 for some initial interval of time $0 \le t \le \omega$. Then for the time interval for which $\omega \le t \le 2\omega$ the solution is, respectively

$$\text{(a)}\ P(t) = \left[a_1/(b+a_2P_0) + \left(\frac{1}{P_0} - \frac{a_1}{b+a_2}P_0\right) e^{-(b+a_2P_0)(t-\omega)}\right]^{-1}$$
$$\text{(b)}\ P(t) = \exp\left\{\frac{b+a_2 \log P_0}{a_1}(1 - e^{-a_1(t-\omega)}) + e^{-a_1(t-\omega)} \log P_0\right\} \tag{8}$$

The equilibrium levels in the absence of fishing for the two cases are easily calculated to be, respectively

$$\text{(a)}\ P_\infty = \frac{b}{a_1 - a_2}$$
$$\text{(b)}\ P_\infty = e^{\frac{b}{a_1 - a_2}}. \tag{9}$$

In both instances if the initial level P_0 is constant for an interval of length ω at the equilibrium level, the population remains at that level. If the initial level is below equilibrium the population increases toward and may exceed equilibrium while the opposite is true if it is initially above equilibrium.

An alternate form for these equations may be obtained by integrating both sides over a unit time interval, i.e. the smallest time interval for which data exists:

$$\text{(a)}\ \log \frac{P(t+1)}{P(t)} = b - a_1 \bar{P}(t) + a_2 \bar{P}(t-\omega)$$
$$\text{(b)}\ \log \frac{P(t+1)}{P(t)} = b - a_1 \int_t^{t+1} \log P + a_2 \int_{t-\omega}^{t-\omega+1} \log P. \tag{10}$$

Here $\bar{P}(t)$ in the first equation denotes the average of P over the time interval from t to $t+1$. The equations in this form are often easier to use in trying to fit data.

The type of behavior expected over a long period of time of the solutions to these equations varies considerably with changes in the coefficients b, a_1, and a_2. In general, solutions to either equation will oscillate finitely about the equilibrium level and will approach that level as time increases sufficiently. However, there are exceptions as illustrated by a number of hypothetical examples in Appendix 1. Their growth curves are given in Fig. 2.

Effects of fishing on the equations — The introduction of fish cropping has the effect of changing

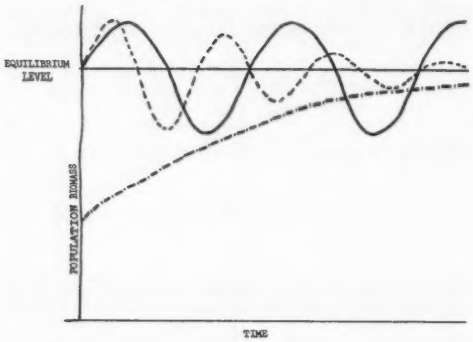

FIG. 2. Three possible growth curves of population biomass for a delay-differential equation model.

the coefficient b and thus decreasing the equilibrium level. It may be introduced into the equation as

$$\text{(a)}\ \frac{1}{P}\frac{dP}{dt} = b - a_1P(t) + a_2P(t-\omega) - qf(t)$$
$$\text{(b)}\ \frac{1}{P}\frac{dP}{dt} = b - a_1 \log P(t) + a_2 \log P(t-\omega) - qf(t) \quad (11)$$

where $f(t)$ is the fishing effort and q the "coefficient of catchability." This coefficient as well as the coefficient b, a_1, and a_2 are determined from data for abundance and fishing effort. There are a number of ways to choose the best values of these coefficients to fit the data. One way which avoids approximation is first to integrate the equations over the smallest time interval for which data exists thereby obtaining equations similar to (10), but including the fishing mortality term:

$$\text{(a)}\ \log \frac{P(t+1)}{P(t)} = b - a_1\bar{P}(t) + a_2\bar{P}(t-\omega) - q\bar{f}(t) \quad (12)$$
$$\text{(b)}\ \log \frac{P(t+1)}{P(t)} = b - a_1 \int_t^{t+1} \log P + a_2 \int_{t-\omega}^{t-\omega+1} \log P - q \int_t^{t+1} f$$

where $\bar{P}(t)$, $\bar{P}(t-\omega)$, and $\bar{f}(t)$ denote the averages of the corresponding levels during this time interval. The coefficients are then determined using standard multiple regression procedures. More details as well as a simplified method are given in Appendix 2.

In either case one is able to calculate the equilibrium level of fishing effort with respect to population. It is

$$\text{(a)}\ b - a_1P(t) + a_2P(t-\omega) - qf = 0.$$
$$\text{(b)}\ b - a_1 \log P(t) + a_2 \log P(t-\omega) - qf = 0. \quad (13)$$

No single curve of fishing effort vs. population under equilibrium conditions can be plotted. Rather one obtains a family of such curves, one for each level of $P(t-\omega)$. However, and surprisingly, one can still find the point of maximum sustained yield. The yield is given by $y = qPf$. By multiplying each term in the first equation by f one obtains an equation involving yield:

$$\text{(a)}\ bf - a_1Pf + a_2P(t-\omega) - qf^2 = 0 \quad (14)$$

or

$$\text{(a)}\ bf - a_1y/q + a_2fP(t-\omega) - qf^2 = 0. \quad (15)$$

This can be solved for yield as a function of fishing effort and delayed population. Now the delayed population is independent of the current fishing effort and thus it is possible to differentiate y with respect to f, and set the result equal to zero to obtain the level of fishing effort which maximizes equilibrium yield. It is

$$\text{(a)}\ b + a_2P(t-\omega) - 2qf = 0. \quad (16)$$

This is now combined with equation (13) to obtain the condition for maximum sustained yield:

$$\text{(a)}\ a_1P - qf = 0. \quad (17)$$

However, from the standpoint of regulations, equation (16) is more important since it enables one to calculate the optimum level of fishing effort from previous population figures.

In the case of model (b), it is necessary to proceed a little differently. Since $y = qPf$, the derivative $\frac{1}{q}\frac{dy}{df}$ is equal to $f\frac{dP}{df} + P$. The maximum value of equilibrium yield is attained when this derivative is equal to 0. Thus it is attained when

$$\text{(b)}\ \frac{dP}{df} = -\frac{P}{f}. \quad (18)$$

The differentiation of (13b) with respect to f yields

$$\text{(b)}\ \frac{-a_1}{P}\frac{dP}{df} - q = 0$$

which when substituted into (18) leads to the level of f which maximizes yield:

$$\text{(b)}\quad a_1 - qf = 0. \tag{19}$$

Both equations (17) and (19) may be solved for that level of fishing effort f_m at which sustained yield is maximized:

$$\begin{aligned}\text{(a)}\quad f_m &= \frac{a_1 P}{q} = \frac{b+a_2 P(t-\omega)}{2q}\\ \text{(b)}\quad f_m &= \frac{a_1}{q}.\end{aligned} \tag{20}$$

These levels should be compared to those obtained for the corresponding instantaneous models given in equation (3). In equation (20a), in particular, the optimum fishing effort depends on the level of the spawning population ω years before. Because this spawning population may not have been at the equilibrium level, the optimum fishing effort will change from year to year, and a fishery manager would have to set variable limits to the catch.

Models with additional delay terms — In the two previously discussed models we have supposed that rate of growth depends on the population at a single previous time. Quite often it may depend on the population at a number of different previous times. This would occur for example in a cannibalistic species that eats its young. It may also be that the age at first spawning is not known exactly so that ω cannot be chosen with precision.

Both difficulties can be resolved by introducing additional delay terms in the equations.

$$\begin{aligned}\text{(a)}\quad \frac{1}{P}\frac{dP}{dt} &= b-a_1 P(t)+a_2 P(t-\omega)+a_3 P(t-2\omega)+\ldots\\ &\quad +a_{m+1}\ P(t-m\omega)-qf\\ \text{(b)}\quad \frac{1}{P}\frac{dP}{dt} &= b-a_1 \log P(t)+a_2 \log P(t-\omega)+\ldots\\ &\quad +a_{m+1}\log P(t-m\omega)-qf.\end{aligned} \tag{21}$$

The parameters $b, a_1, a_2, \ldots, a_{m+1}$ and q may be obtained by fitting the equations to population and fishing intensity data using standard multiple regression techniques. However, only those delay terms which significantly affect growth should be included. This can be accomplished by using a step by step regression procedure which tests each term for significance before it is included.

The two models (21), though more complex in appearance, are no more difficult to apply. In fact, the level of fishing intensity which maximizes sustained yield is again given by the formula (20). If a step by step regression procedure is used, the nonsignificant variables which may include the current population biomass or even fishing intensity are eliminated. This turned out to be the case in six of eight species studied by Walter and Hogman (1971).

In a multi-species fishery, of course, additional terms reflecting interspecies relationships need to be introduced. This was done for a model of the first type in the reference cited above.

There seems to be no a priori reason for choosing either of these two models over the other. The second is easier to analyze mathematically because it may be converted into a linear equation by means of a change of variable. Silliman (1971) has pointed out that the second model without the delay terms gives a better fit than the first to data from laboratory experiments. However, a final determination of which model to use depends on the biological characteristics of a given population.

Appendix 1.

SOME HYPOTHETICAL EXAMPLES

Example 1. $\dfrac{P'}{P} = \dfrac{3\pi}{4}(\sqrt{2} + 1 - \log P(t) - \sqrt{2}\ \log P(t-1))$

has an equilibrium level of $P_\infty = \dfrac{1+\sqrt{2}}{e^{1+\sqrt{2}}} = e$ and a solution

$$P(t) = e^{1+\sin\frac{3\pi}{4}t}$$

which oscillates finitely about P_∞ but doesn't approach it.

Example 2. $\dfrac{P'}{P} = b - 2\log P(t) + e \log P(t-1)$

has an equilibrium level of $P_\infty = e^{\frac{b}{2-e}}$ and a solution given by

$$P(t) = e^{\frac{b}{2-e}} e^{e^{-t}}$$

which approaches the equilibrium level monotonically (without oscillating) as t approaches infinity.

Example 3. $\dfrac{P'}{P} = (1 - P(t) + 2P(t-1))$

has an equilibrium level of $P_\infty = \dfrac{1}{1-2} = -1$, which is negative. The solution in the interval from $t = 1$ to

$t = 2$ assuming a constant initial value of P_0 for $0 \leq t \leq 1$ is given by

$$P(t) = 1/[(1/(1+2P_0)) + (1/P_0 - 1/(1+2P_0))\, e^{-(1+2P_0)(t-1)}]$$

which is an increasing function no matter what the initial value of P_0 is. In this case a condition of unstable equilibrium exists.

Example 4. $$\frac{P'}{P} = \frac{\pi e}{2} - 1 - \log P(t) + \frac{\pi e}{2} \log P(t-1)$$

has an equilibrium level of $P_\infty = e^{-1}$. A solution is given by

$$P(t) = e^{-1}\, e^{e^{-t}} \sin \frac{\pi}{2} t$$

which oscillates finitely about the equilibrium level while gradually approaching it.

Appendix 2.

Computing the Values of the Parameters in the Time-lag Equations

In studying a fishery by means of any of the simple models, often the most difficult and onerous task is that of compiling catch per unit effort and fishing effort data. We shall lightly gloss over this task and assume we have catch per unit effort data $P_1, P_2, \ldots, P_n$ and fishing effort $f_1, f_2, \ldots, f_n$ for both over a period of n years. Either one may or may not be expressed as a percent by dividing by some standard population and fishing effort. The analysis is the same. It should be observed that we have used the same symbol P to denote both catch per unit effort and population biomass. Since the two quantities are proportional, this seems less confusing than the introduction of another symbol.

We shall present only the procedure for the first type of time-lag model. Again ω represents the number of years lag from spawning to recruitment. We shall assume that P_i and f_i represent the catch per unit effort and fishing effort at the end of the i^{th} year and that this is the same as the average over that year. We start with equation (12a) expressed at the end of the i^{th} year:

$$\log_e \frac{P_i}{P_{i-1}} = b - a_1 P_i + a_2 P_{i-\omega} - q f_i. \qquad (21)$$

This is the equality we should expect if our equation describes the growth exactly. Then the parameters b, a_1, a_2, and q may be obtained by solving any system of four equations given by (21) at four distinct times. Unfortunately this is rarely, if ever, the case, and the parameters must be chosen in such a way as to make the difference between the left and right hand sides of (21) as small as possible. This can be accomplished by using the well-known normal equations to obtain the best (least squares) fit. This procedure is known to many biologists but almost requires the use of a computer to make the necessary calculations.

An alternate procedure is first to calculate the parameters for the instantaneous model, either graphically or numerically. This gives us numbers b', a', and q' such that

$$\log_e \frac{P_i}{P_{i-1}} - (b' - a'P_i - q'f_i) = g_i \qquad (22)$$

is as small as possible. We then choose parameters b'' and a'' again either graphically by plotting g_i vs. $P_{i-\omega}$ or numerically in such a way that

$$g_i - (b'' + a''P_{i-\omega}) \qquad (23)$$

is as small as possible. This gives an approximation (though not the best one) to our desired parameters in the form

$$b = b'' + b'', \quad a_1 = a', \quad a_2 = a'', \quad q = q'.$$

Table 1. Optimum levels of fishing effort in the California sardine fishery for representative years from 1932 to 1954 as estimated by the instantaneous and time-lag models.

Year	f opt (Inst.)	f opt (Time-lag)	f actual
1934	1175	1800	800
1939	1175	940	1400
1944	1175	940	1500
1949	1175	810	950
1954	1175	310	550

Table 2. Optimum levels of fishing effort in the western Atlantic yellowfin tuna fishery for selected years from 1956 to 1966 as estimated by the instantaneous and time-lag models.

Year	f opt (Inst.)	f opt (Time lag)	f actual
1960	9.94×10^6	12.5×10^6	5.3×10^6
1961	9.94×10^6	5.7×10^6	2×10^6
1963	9.94×10^6	6×10^6	13×10^6
1966	9.94×10^6	6.4×10^6	9.1×10^6

If, as is often the case, we are interested only in the optimum level of fishing effort, we can skip the second step. Since this level is given by

$$f_{opt}(t) = \frac{a_1 P(t)}{q}$$

only the parameters a_1 and q obtained in the first step are needed. This has the added advantage of giving us an easy comparison between the time-lag and the instantaneous models for fisheries to which the latter has been applied.

Example 1. *The California sardine from 1932 to 1954 (Data from Fox)* — The parameters q and a_1 as calculated by Fox were $q = 7.86 \times 10^{-4}$, $a_1 = 1.78 \times 10^{-3}$. The optimum level of fishing for the instantaneous model is given by the same value for all years and was

$$f \text{ opt} = \frac{a_1 P_\infty}{2q} = 1175.$$

For the time-lag model the optimum level depends on the population and thus varies from year to year. The results of calculations for some representative years are given in Table 1.

It should be observed that both models agree in the years 1934, 1939, and 1944 but disagree in 1949 and 1954 as to whether the fishing was excessive. In fact the fishing was excessive according to the time-lag model for every year from 1936 to 1954. This may help to explain the decline of the sardine in later years.

Example 2. *Lake trout in Green Bay, Lake Michigan from 1929 to 1944 (Data from Walter and Hogman)* — The calculated parameters were $a_1 = 2.3 \times 10^{-3}$, $q = 7.5 \times 10^{-4}$. The value for f opt was 194 in the instantaneous model. The values for f opt in the years from 1929 to 1944 given by the time-lag model varied from 135 in 1944 to 460 in 1937. The actual fishing effort exceeded the optimum in only one year, 1931, according to either model. This reinforces the belief that over fishing was not the cause of the decline of the lake trout in the late 1940s.

Example 3. *Western Atlantic yellowfin tuna from 1956 to 1966 (Data from Fox)* — The calculated parameters were $a_1 = .347 \times 10^2$ and $q = 1.21 \times 10^{-7}$. The value for f opt was 9.94×10^6 for the instantaneous model. For the time-lag model the values are given in Table 2.

The two models agreed for every year except 1966 in which the estimate for the instantaneous model indicated that the actual level of fishing was below the optimum, but the estimate for the time-lag model indicated that it was above. On the basis of the latter model we would expect a continuing decline in the population while in the former we would not.

BEVERTON, R. J. H., AND S. J. HOLT. 1957. *On the dynamics of exploited fish populations*. Fish. Invest. London Ser. 2(19): 533 p.

FOX, W. W., JR. 1970. An exponential surplus yield model for optimizing exploited fish populations. Trans. Amer. Fish. Soc. 99: 80–88.

PELLA, J. J., AND P. K. TOMLINSON. 1969. A generalized stock production model. Bull. Inter-Amer. Trop. Tuna Comm. 14: 421–496.

RICKER, W. E. 1958. Handbook of computations for biological statistics of fish populations. Fish. Res. Board Can. Bull. 119: 300 p.

SCHAEFER, M. B. 1968. Methods of estimating effects of fishing on fish population. Trans. Amer. Fish. Soc. 97: 231–241.

1954. Some aspects of the dynamics of population important to the management of the commercial marine fisheries. Bull. Inter-Amer. Trop. Tuna Comm. 1: 27–56.

SILLIMAN, R. P. 1971. Advantages and limitations of "simple" fishery models in light of laboratory experiments. J. Fish. Res. Board. Can. 28: 1211–1214.

WALTER, G., AND W. HOGMAN. 1971. Mathematical models for estimating changes in fish populations with applications to Green Bay. Proc. 14th Conf. Great Lakes Res. 170–184.

WANGERSKY, P. J., AND W. J. CUNNINGHAM. 1957. Time lag in population models. Cold Spr. Harbor Symp. Quant. Biol. 22: 329–338.

Observations on the Cestode *Eubothrium salvelini* in Juvenile Sockeye Salmon (*Oncorhynchus nerka*) at Babine Lake, British Columbia

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SMITH, H. D. 1973. Observations on the cestode *Eubothrium salvelini* in juvenile sockeye salmon (*Oncorhynchus nerka*) at Babine Lake, British Columbia. J. Fish. Res. Board Can. 30: 947–964.

Infection incidence among 15 brood years of smolts in the period 1952–71 was estimated from smolt and underyearling samples, and among late run fish from the main part of Babine Lake ranged from 6 to 45% (average 26%). In early run fish from the North Arm–Nilkitkwa area it ranged from 5 to 46% (average 22%) in 10 years during 1958–71.

Infection intensity changed substantially during 1967; but about one fish in eight, or 200,000 individuals in the early run, carried parasite loads $\geqslant 10\%$ of fish weight. Mature *E. salvelini* (Schrank 1790) averaged 32 mg in weight and 34 mm in length during a period of high incidence and intensity of infection in 1967.

Although no proof was found of direct mortality caused by *E. salvelini*, indirect effects such as inferior growth, poor swimming performance, or aberrant behavior due to large cestode loads may increase host susceptibility to death from other causes.

Changes in relative abundance of the zooplankton intermediate host, and midsummer shifts in feeding preferences of young sockeye likely account for increasing infection incidence in June and July, and stable incidence thereafter.

SMITH, H. D. 1973. Observations on the cestode *Eubothrium salvelini* in juvenile sockeye salmon (*Oncorhynchus nerka*) at Babine Lake, British Columbia. J. Fish. Res. Board Can. 30: 947–964.

Au cours de la période 1952–71, l'auteur a estimé sur des échantillons de smolts et de saumons de moins d'un an l'incidence d'infestation dans 15 classes annuelles, et a constaté que, chez les saumons de montaison tardive de la partie principale du lac Babine, l'incidence varie de 6 à 45% (moyenne 26%). Chez les saumons de montaison hâtive de la région bras Nord-Nilkitkwa, l'incidence varie de 5 à 46% (moyenne 22%) au cours de 10 années entre 1958 et 1971.

L'intensité d'infestation a changé substantiellement au cours de 1967; mais environ un poisson sur huit, soit 200,000 individus de la montaison hâtive, portaient une charge de parasites $\geqslant 10\%$ du poids corporel. Les *E. salvelini* (Schrank 1790) mûrs pesaient en moyenne 32 mg et mesuraient 34 mm de longueur lors d'une période de haute incidence et de forte intensité d'infestation en 1967.

Bien qu'on n'ait pas trouvé de preuve de mortalités directes causées par *E. salvelini*, des effets indirects tels faible croissance, pauvre performance natatoire ou comportement aberrant, résultant d'une forte infestation de cestodes, peuvent rendre l'hôte plus vulnérable à d'autres facteurs de mortalité.

Les variations d'abondance relative de l'hôte intermédiaire zooplanctonique et le changement, à la mi-été, des préférences alimentaires des jeunes saumons nerka sont sans doute responsables d'une plus grande incidence d'infestation en juin et juillet, incidence qui demeure stable par la suite.

Received October 10, 1972

THE cestode *Eubothrium salvelini* (Schrank 1970) is a common intestinal parasite of salmonids in northern latitudes (Wardle and McLeod 1952; Hoffman 1967; Yamaguti 1959; Bykhovskaya-Pavlovskaya et al. 1964). It occurs across the entire east–west axis of northern USSR (Dogiel 1958), and in Canada is common in trout and char (MacLulich 1943; Bangham and Adams 1954). Margolis (1963) found *E. salvelini* in samples of sockeye salmon juveniles from three North American rivers,

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and believes larval *Eubothrium* species from at least three other rivers were probably *E. salvelini* (personal communication). Although Margolis did not find the parasite in all sockeye populations sampled, his data indicate a western North American range from at least the Columbia River in Washington State to Egegik River in Bristol Bay, Alaska — virtually throughout the north–south sockeye range.

E. salvelini was believed to cause mortality among wild and hatchery-produced juvenile salmonids in Canada over 30 years ago (Wardle 1933) and Dombroski (1955) found the parasite in more than one-third of Babine Lake sockeye smolts sampled in 1952 and 1953, and infected smolts averaged smaller than noninfected ones. More recently (in 1958) L. Margolis and (in 1963) L. Margolis and N. P. Boyce (personal communications) found a similar incidence of infection among Babine sockeye smolts and underyearlings (the latter during the summer prior to migration) and at the lake outlet annually from 1961–71 I have observed small, listless smolts with distended bellies, and often with cestodes trailing from the anus. In 1967, high water forced a catch of smolts to swim overnight against a 1 knot current in a holding pen. Many heavily infected smolts died during this period while noninfected ones appeared not to suffer ill effects.

In view of these observations, a study was undertaken to assess effects of the parasite on Babine Lake sockeye. Results thus far suggest that heavily infected fish fatigue more rapidly than noninfected ones of similar size when tested together in a swimming performance tunnel, and that heavy infection could be serious for afflicted fish (Smith and Margolis 1970).

This paper summarizes information gathered at Babine Lake and in our laboratory from 1952–71. It indicates the general incidence and intensity of infection in Babine Lake sockeye juveniles and along with apparent effects of the parasite on host fishes is the basis for an hypothesis to explain some aspects of the dynamics of infection. Detailed studies of the parasite's life history and measurements of certain differences between infected and noninfected fish are continuing and will be reported separately.

Study Area and its Juvenile Sockeye Population

Babine Lake is 155 km long and 49 km^2 in surface area (Fig. 1). It drains northward through a narrow North Arm (10 km long), short Upper Babine River, Nilkitkwa Lake (3 km long), and thence 400 km to the ocean via the Lower Babine and Skeena rivers. Sockeye fry migrate from tributary streams in May and June, and reside 1 year in the lake as "underyearlings." About 95% emigrate to sea as "smolts" at age 1; the remainder stay an additional year in fresh water and migrate at age 2.

Smolts are probably widely distributed in the lakes prior to onset of migration. Sonar "targets" believed to be smolts move northward, particularly along shorelines in many parts of the lake in late May and early June (C. Groot personal communication). Others caught in a trap inshore and tagged at Sandspit Point (Fig. 1) in June 2, 1961 were later recovered at the outlet (Johnson and Groot 1963). These authors thought annual smolt migrations began at about the same time throughout the system. Foerster (1968) suggested that in many systems migration may begin about coincident with ice breakup on the lakes.

The smolt run at the lake outlet is bimodal. It includes an early run of migrants from the combined

Fig. 1. Babine Lake showing places named in the text. Regions north and south of the junction with Morrison Arm are termed North Arm and Main Lake, respectively.

North Arm of Babine Lake and Nilkitkwa Lake nursery areas, and a late run of migrants from Morrison Arm and the southern lake basins of Babine (the Main Lake). Because ice usually leaves the Main Lake several weeks earlier than from the more northerly waters, the two modal groups often migrate under quite different thermal regimes: whereas early fish travel for only a few days at temperatures not greatly changed since winter, late migrants are 3–4 weeks en route while surface temperatures at the outlet rise from an initial 5 C to about 9 C.

Little exchange of juveniles is believed to occur between the two major nursery areas but subpopulations within them (composed of fish from distinct spawning grounds) probably mix extensively (McDonald 1969).

Babine Lake kokanee (*Oncorhynchus nerka kennerlyi*), a nonanadromous form of sockeye, are indistinguishable from anadromous sockeye at early life stages. Those believed to be 3 years and older have not been found infected, but a few younger ones were. Rainbow trout infected with *E. salvelini* have been captured in Nilkitkwa and Babine lakes, but incidence in these and perhaps other resident fishes is unknown.

Life Cycle

Vik (1963) found that the cyclopoid copepod *Cyclops strenuus* is the natural intermediate host of *E. salvelini* in Norway. Vik's findings are supplemented here with information from current studies at Babine Lake.

The cestode anchors itself in a pyloric caecum by drawing portions of host epithelium into one or both of paired bothria. Long worms may trail into the host intestine or be convoluted and penetrate into adjoining caeca. Egg production is likely anapolytic, i.e. eggs are discharged into the host gut and thence reach the water. They were observed to sink slowly in flasks of Babine Lake water at about 7.5 C. The intermediate host at Babine may be exclusively *Cyclops scutifer*[1] which according to Narver (1970) is an important food item of Babine sockeye juveniles. Advanced stages of *C. scutifer* were successfully infected with eggs of *E. salvelini* in the laboratory at Nanaimo, but not the sympatric calanoid *Diaptomus* sp. (N. P. Boyce personal communication).

When ingested by a copepod, the eggs hatch and the liberated oncospheres employ paired hooks to incise the gut wall and enter the hemocoel. The larval stage then develops, but in common with other pseudophyllidean cestodes, it likely cannot infect a definitive host until cercomer formation — perhaps 2–3 weeks later (Smyth 1963). If the infected copepod is eaten by a susceptible fish the larval worm may eventually reach a pyloric caecum where it will develop to the adult stage, and presumably remain for the rest of its life. There is no evidence that an intermediate host fish is implicated.

The life span is not definitely known. I found no infections in four samples each of 50 jack sockeye (adult males having spent 1 year in each of freshwater and saltwater habitats) returning to Babine Lake in early August 1968 and 1969 from late smolt runs having infection levels of 26 and 35%, respectively. This suggests that the parasites died (or perhaps were eliminated) in fewer than 25 months while at sea unless the bulk of infected fish perished or matured at a greater age. However, in 1968 I transported several hundred infected smolts from Babine Lake to the Pacific Biological Station and placed equal numbers in separate freshwater and saltwater holding tanks and after 14 months some in each tank were still infected. If the cestodes became established in those fish shortly after they entered the lake as fry, a life span of at least 24–26 months is assured. This suggests, incidentally, that worms may occasionally survive to the jack stage of the hosts despite the conflicting evidence of 1968 and 1969. In any event, the maximum life span thus far demonstrated in sockeye salmon is considerably less than the "2 years, 7 months" reported in rainbow trout by Hoffman (1967).

Methods

Smolt Samples

Smolts were obtained from runs migrating seaward from Babine Lake in May and June. Beginning daily in 1950, smolts were captured in fixed traps and transferred to holding pens where samples of 100 or more fish were randomly dipnetted. The samples were assumed to be representative of the run passing in the previous 24-hr period and were used to estimate daily infection incidence in late runs only from 1954 to 1956, and in both early and late runs from 1966 to 1971.

In addition, in 1966 and 1967 fork lengths (mm), wet weights (g), and worm counts were obtained from every sampled fish; however, in 1967 only, a number of live worms were weighed and measured. Processing details in the several years were as follows:

1966: Samples were stored in 10% formalin several months. Intestinal tracts and swollen pyloric caeca were

[1]Gunnerød (1971) cites a personal communication from C. Elgmork to the effect that *C. strenuus* and *C. scutifer* are indistinguishable at early life stages. Gunnerød also raises the interesting possibility that the cyclopoid known at Babine Lake and Iliamna Lake, Alaska as *C. scutifer* is in reality *C. columbianus* but concedes that further study is required on this point.

then opened and visible worms were removed and counted under a binocular miscroscope. Data errors attributable to overlooking very small worms in caeca are considered below. Early, late, and annual incidence were calculated by weighting sample incidence to daily smolt abundance and summing over appropriate periods.

1967: During May 17–June 12, 20–50 fresh smolts were processed daily within a few hours after removal from traps. Other, identified by fin marks imposed on fry at Fulton River on the Main Lake, were preserved and processed as in 1966, but their numbers varied according to composition of the daily runs.

All fresh fish were killed in a mild overdose of 2 phenoxyethanol and 20–30 were opened and cestodes were removed, as far as possible, intact. The remainder were opened, the presence or absence of cestodes was noted, then specimens were discarded. Daily infection incidence was calculated from the total sample.

Live worms were measured to the nearest mm on a plastic-covered measuring board directly after removal from the host. Each was gently extended as far as possible by cradling it anteriorly in the bend of an offset steel probe, then sliding the probe back under the worm to straighten it and remove twists. Measurements were accepted only if worms did not immediately contract, but were repeated as often as necessary. Worms were weighed to the nearest 10 mg after uniform blotting to remove excess moisture and foreign materials. Time limitations necessitated changes in kinds of measurements during the run as follows:

	Measurement			
	Length		Weight	
Smolt run[2]	Individual	Aggregate	Individual	Aggregate
May 17–22	×	×		
May 23–27	×	×	×	×
May 28–June 12				×

[2]After May 27 mean cestode weights were obtained by

$$\frac{\text{aggregate weight}}{\text{worm numbers}}$$

Infection incidence for the entire run was calculated as in 1966. Incidence for the Fulton population was determined by weighting the daily sample incidence by estimates of daily output used by McDonald (1969, Table II).

Parasite load is defined here as

$$\text{Load} = \frac{w(100)}{W - w}$$

where W, and w are wet weights of fish and worms, respectively. Load was calculated for all infected smolts from May 17–June 12 and grouped to early and late runs. Aggregate worm weight without regard to host size, is termed "worm burden" in this paper.

Distributions of load and infection intensity were skewed, and arithmetic means and variances were generally proportional in both worm lengths and weights. Means were therefore transformed to logarithms to permit statistical comparisons. Geometric means (antilogs of means of transformed data) which are slightly smaller than arithmetic means, were plotted for visual comparison but since much of the pertinent literature deals only in arithmetic means, that measure is included in several instances as well.

1968–71: All smolts in daily samples of 70–150 were examined to assess daily infection incidence and weighted to daily fish abundance. Occasional cestode counts an measurements during 1968–71 revealed no striking differences from those of 1966–67, so the latter are thought to be quite representative of all recent years.

Underyearling Samples

Underyearlings from townet catches were used to assess density and distribution of sockeye during their first summer in Babine Lake (Johnson 1956) and from seine catches for studies of distribution, growth, and survival of marked Fulton River juveniles (McDonald 1969).

Presence or absence of cestodes in underyearlings was determined as described for preserved smolts from four separate years as follows: first, infection incidence in townet samples of September–October, 1959 made available by L. Margolis and N. P. Boyce (personal communication) was supplemented with all available June–August data of that year; next, 1960 and 1961 townet samples were selected to give a broad temporal and spatial representation and subsamples of 30 fish from each were examined for parasites; finally, 358 Fulton River fish seined in 1966 were added. Results were grouped to three lake regions as follows:

Region 1 — North Arm–Nilkitkwa (early run nursery area);

Region 2 — Morrison Arm and Main Lake to Sandspit Point;

Region 3 — Main Lake; Sandspit Point to Lakehead.

Error Due to Nondetection of Cestodes

Some very small worms in smolts were occasionally overlooked because only discolored or distended pyloric caeca were examined microscopically. Such worms, usually less than 10 mm long, were likely acquired during the spring of migration. Apparently, they were uncommon and in view of their small size seemed unlikely to affect appreciably either counts and measurements or the well-being of juveniles in fresh water.

Smolts infected with larger worms presumably acquired the previous spring were readily identified. For example, when whole fresh smolts from the early 1967 run and preserved ones from the entire 1969 run were judged infected or not on the basis of external appearance (size, abdominal swelling, etc.), then examined microscopically, the following results were obtained:

Smolt Category	1967 Fresh smolts Correct No. (%)	1967 Fresh smolts Incorrect No. (%)	1969 Preserved smolts Correct No. (%)	1969 Preserved smolts Incorrect No. (%)
Infected	68(88.3)	9(11.7)	945(98.1)	18(1.9)
Noninfected	157(94.0)	10(6.0)	1164(94.1)	73(5.9)
Both	225(92.2)	19(7.8)	2109(95.9)	91(4.1)

Assessments were 92 and 96% accurate in 1967 and 1969, respectively, and because incorrect assessments partially offset one another within years, net error in assessing annual infection incidence was appreciably lower. This test suggests that noninfected smolts, as well as those believed to have acquired infection in the year prior to host migration were seldom incorrectly categorized.

Some small worms are doubtless overlooked in underyearlings, but most appear to become established in spring and early summer (see below) so errors from this source should be small in samples taken thereafter.

Results

Incidence of Infection

Incidence was estimated for early, late, and total runs in 10 separate years (Table 1) and most of the latter estimates were of similar magnitude (about 20–30%) despite being measured at the smolt stage in some years and underyearling stage in others. Early and late run estimates tended to fluctuate together, but late run estimates were higher than those of early runs in 7 of the 10 years.

Among 15 years of late runs, 10 had rather similar high incidence (mean 32%, range 25–45%) while the remainder were about one-third as large (mean 12%, range 6–18%), and the latter were clustered in consecutive years — i.e. 1955–57 and 1970–71.

Measurements were not made for any single year class at both underyearling and smolt stages of the total lake population, but within the Fulton River substock it was 60% among underyearlings in 1966, and 59% among smolts in 1967.

Daily incidence — Estimated numbers of infected and noninfected fish in daily smolt runs from 1966–71 are shown in Fig. 2. Several features were common to most years: 1, infected fish were present in all daily runs but constituted highly variable proportions of daily totals (e.g. 8–72% in 1966); 2, infection incidence was usually inversely related to number of migrants (especially in 1966 and 1967); 3, incidence was usually at or near seasonal maxima in the last few days of both early and late runs.

Table 1. Annual incidence of infection of sockeye salmon smolts and underyearlings with *Eubothrium salvelini*. Underyearling data listed by year of migration as smolts; 1966–71 data weighted to abundance.

Year of smolt migration	Region: North Arm-Nilkitkwa (early run) Sample size	North Arm-Nilkitkwa (early run) Infection rate (%)	Main Lake (late run) Sample size	Main Lake (late run) Infection rate (%)	Entire Babine Lake system Sample size	Entire Babine Lake system Infection rate (%)	Source of data
1952			1654	33			Dombroski (1955)
1953			1234	38			Dombroski (1955)
1954			972	13			Annual records } Withler and Aro (unpublished)
1955			1977	6			Annual records } Withler and Aro (unpublished)
1956			2041	10			Annual records } Withler and Aro (unpublished)
1958[a]	1465	19	911	25	2376	21	Margolis (unpublished)
1960[a]	1242	46	1052	45	2294	46	Margolis and Boyce (unpublished)
1961[a]	597	32	820	25	1417	28	Tow-net catches of underyearlings
1962[a]	300	30	606	28	906	29	Tow-net catches of underyearlings
1966	2000	25	2100	37	4100	31	Current studies
1967	1900	15	1400	30	3300	21	Current studies
1968	600	20	2400	26	3000	24	Current studies
1969	1400	23	2000	35	3400	29	Current studies
1970	2100	6	3450	14	5550	12	Current studies
1971	1350	5	2650	18	4000	14	Current studies

[a]Estimates based upon incidence among underyearlings in summer and autumn before migration.

Incidence in underyearlings — Markedly different rates of parasite acquisition occurred within each of two sampling periods (Fig. 3) as follows: 1, June–July: incidence varied considerably between years and regions but increased during this period from zero to near it to a maximum of 40%.

Samples were obtained in June in either two or three of the regions in three separate years, but only fish in the Sandspit to Southend region were infected (in 1961 and 1966). In contrast all July samples included infected fish and they indicate a general increase in incidence from north to south; 2, August–October: incidence did not rise appreciably, and by October was stable throughout the lake. Greatest October range in incidence among regions in any year was only 6% (in 1959). In other years it was less than 4%.

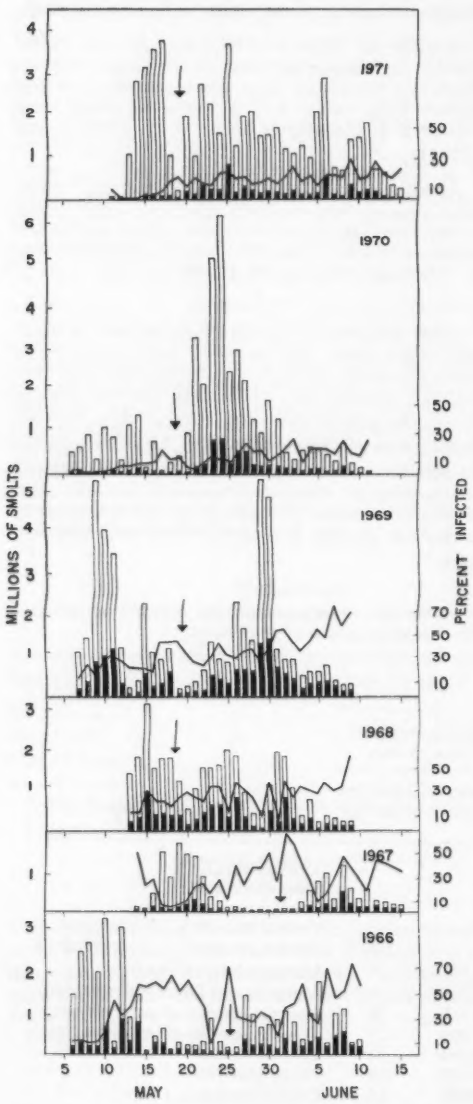

FIG. 2. Estimated numbers of smolts and infection incidence in daily smolt migrations 1966–71. Open portions of bars show numbers noninfected, solid portions show numbers, and lines percent infected with *E. salvelini*. Arrows divide early and late runs.

INTENSITY OF INFECTION

Table 2 shows the range and both arithmetic and geometric mean intensity of infection in early, late, and total smolt runs of 1966 and 1967. Minimum and maximum numbers of cestodes were similar, and highest means occurred in early runs in both years. In Table 3 arithmetic and geometric means of daily infection intensity in 1967 are shown along with log-transformed values for means and standard deviations. (The latter avoids two different arithmetic intervals for the SD of geometric means.) Cestode counts and their variability among and within days declined abruptly after June 3. These intensities agree well with occasional counts taken in other years, thus suggesting a rather consistent annual pattern of intensity in Babine smolt runs.

Distribution of cestode counts in 6–7 successive sampling periods in 1966 and 1967 is shown in Fig. 4.[3] Excepting a few fish carrying rather large numbers of cestodes from May 9 to 15, 1967, geometric mean intensities (indicated on the figures by arrows) were highest, and exceptionally large populations were commonest, a few days prior to the ends of the early runs. They then declined until near the ends of the late runs when some increase again occurred. Within sampling periods there was frequently a rather abrupt drop from numbers of fish carrying 3–4 worms to numbers carrying 5–6 or more — particularly in the last two periods of 1967.

LENGTHS AND WEIGHTS OF WORMS

Aggregate weights (worm burdens) shown in the lower half of Fig. 5 were quite variable through-

[3]To assess possible temporal or spatial trends in smolt and parasite populations, some counts and measurements were grouped to standardized "sample periods" of 7 days each. Some variability in lengths of periods results because fish did not run on all days and in all sample periods, and one period each year was divided according to timing of early and late runs.

out the run but suggested two periods of change, followed by a period of stability as follows: (1) May 23–29 — a moderate increase in mean burden from 116 mg, May 23 to 187 mg, May 29; (2) May 30–June 3 — a rapid decline from high to low burdens (likely smoothed somewhat by the presence of appreciable numbers of both early and late run fish); and (3) June 4–12 — a stable burden with mean 81 mg (about half the 166 mg mean of May 23–29).

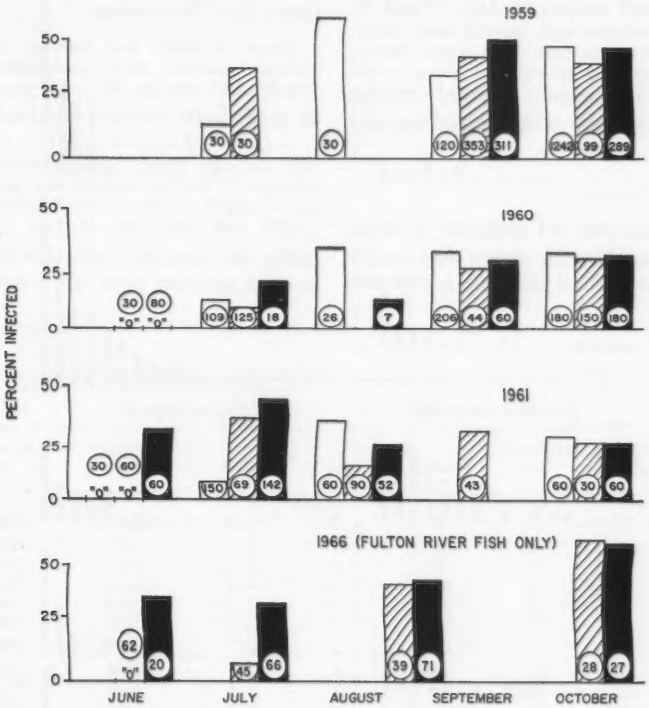

FIG. 3. Incidence of infection of underyearlings by *E. salvelini* in three lake regions 1959–61, and two regions in 1966. (Sample sizes encircled within corresponding bars.)

TABLE 2. Infection intensity in early, late, and total smolt runs of 1966 and 1967.

Year and run	Sample size (infected fish only)	Infection intensity			
		min.	max.	arith. mean	geom. mean
1966 early	388	1	16	3.38	2.48
late	448	1	16	2.73	2.06
total	836	1	16	3.10	2.26
1967 early	173	1	17	4.30	3.14
late	173	1	13[a]	2.82	2.29
total	346	1	17[a]	3.56	2.68

[a]A single fish with 28 small cestodes was omitted from calculations.

The regular upward trend in mean lengths in the upper half of Fig. 3 tends to confirm that worm burdens were increasing during the period of variable weights (May 23–27), and also suggests that this trend had been in effect from the earliest days of sampling. Except for unusually high values associated with small samples on May 17 and 25, individual mean weights and lengths were rather constant over the entire period. A slight decline in individual weights is thought to reflect slight increases in counts of recently acquired cestodes. (Daily sample sizes appear with infection intensity data in Table 3.)

Maturation of Worms

Worm maturation was not examined in detail, but beginning about May 23, worms were usually gravid as indicated by their shedding a profusion of eggs when handled. To illustrate the general size of *E. salvelini* found to respond in this way when taken from Babine Lake smolts, detailed measurements during May 23–27 are summarized in Table 4.

Smolt Size Differences

Figure 6 shows that infected smolts were consistently smaller than noninfected ones. (Fresh lengths and weights of 1967 were first converted to approximate formalin preserved equivalents by $L = L \times 0.95$ and $Wt = Wt \times 1.10$.) The dotted line through means reveals common directional changes in size of infected and noninfected fish in most sample periods. Notable exceptions were May 26–29, 1966 and May 30–June 1, 1967 whereinh ealthy fish decreased and infected fish increased in mean size over those of the preceding period.

Table 3. Numbers of fish examined and daily intensity of infection in parasitized 1967 smolts.

Date	Numbers examined[a]		Infection intensity			
	Infected fish	Worms	AM	GM	Log_{10} Mean	Log_{10} SD
May 17	2	2	1.0	1.0	.0000	.0000
18	2	4	2.0	2.0	.3010	.0000
19	3	6	2.0	1.6	.2007	.3476
20	5	32	6.4	4.8	.6803	.3693
21	11	29	2.6	2.1	.3145	.3239
22	11	36	3.3	2.2	.3465	.3871
23	9	29	3.2	2.7	.4309	.2888
24	12	53	4.4	3.5	.5494	.2653
25	7	24	3.4	3.2	.5055	.1723
26	19	79	4.2	2.9	.4584	.3688
27	12	52	4.3	3.3	.5250	.3530
28	17	90	5.3	4.0	.5995	.3662
29	18	120	6.6	5.1	.7042	.3470
30	18	87	4.8	4.0	.5978	.3022
31	12	40	3.3	2.3	.3531	.3624
June 1	15	62	4.1	3.1	.4906	.3416
2[b]	20	81	4.1	3.4	.5257	.2739
3	20	60	3.0	2.2	.3422	.3194
4	14	30	2.0	1.8	.2653	.1899
5	5	9	1.8	1.6	.2158	.2097
6	8	24	3.0	2.2	.3514	.3445
7	15	42	2.8	2.5	.4033	.1971
8	23	63	2.8	2.2	.3342	.2998
9	19	54	2.8	2.2	.3453	.3041
10	15	41	2.6	2.4	.3798	.1773
11	10	30	3.0	2.6	.4192	.2282
12	24	58	2.4	2.0	.2989	.2661

[a]Most samples consisted of 50 fish.
[b]First day of late run.

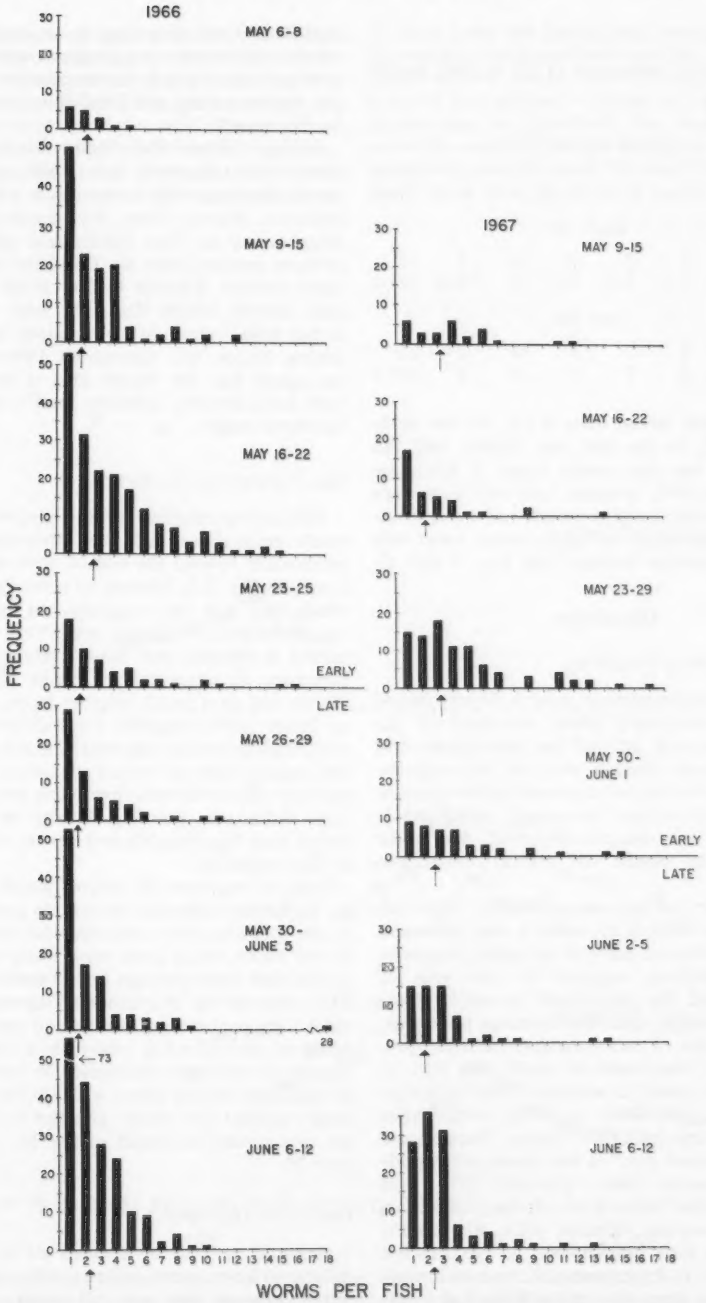

FIG. 4. Intensity of infection of smolts with *E. salvelini* in sample periods of 1966 and 1967. Arrows show geometric mean numbers of worms per infected fish. Periods separated to early and late runs as labelled.

Parasite Load

Parasite load in increments of 5% in 1967 smolts were:

	Load interval (%)							
	<5	5–9	10–14	15–19	20–24	25–29	30–34	Total
	Early run							
No.	97	50	16	5	2	0	1	171
%	56.7	29.2	9.4	2.9	1.2	0	0.6	100.0
	Late run							
No.	157	16	0	0	0	0	0	173
%	90.8	9.2	0	0	0	0	0	100.0

Geometric mean loads were 4.1% in the early run and 1.7% in the late run. Nearly half the infected early run fish carried loads $\geqslant 5\%$; one in eight carried 10% or more. Late run loads were all $< 10\%$. Smaller loads in the late run are attributable to a combination of larger mean smolt size and smaller parasite burdens (see Fig. 5 and 6).

Discussion

Annual Infection Incidence

Intermittent measurements over a 20-year period suggest that commonly about one-third of the population becomes infected by midsummer and that this incidence persists until the fish emigrate as smolts. Two periods of exceptionally low infection (see Table 1) show that this usually rather stable infection level can become disrupted, and lower levels may then persist for several consecutive years.

Higher worm counts among Main Lake fish most years are believed to reflect a real difference in incidence in the two parts of the system, however, occasional infections acquired in the year of migration would be overlooked in early (North Arm) fish and might bias the estimates somewhat.

Initial infection of underyearlings occurred progressively later from south to north (see Fig. 3). This is probably geared to warming trends in surface waters of the lake which normally occur latest in the North Arm–Nilkitkwa region. Temperature plays an important part in the timing of cestode infections (Awachie 1966; Kennedy 1971) and laboratory studies show that warming of waters supporting copepods infected with *E. salvelini* also speeds up their development to the infective stage for fish (N. P. Boyce personal communication).

Infection may occur first in the Main Lake near shore. Guttowa (1963) in discussing uptake of *Diphyllobothrium latum* eggs by copepods in Finland refers to lakeshore areas as places where "ice thaws most quickly in spring, the temperature rises quickly and there is waving and good illumination necessary for hatching."

Among Fulton fish higher incidence in the south than in the north Main Lake region does not necessarily mean the former is a prime focus of infection. Fulton River fry disperse southward after leaving the river (McDonald 1969), so some of those sampled near the Lakehead in June could have become infected farther north but carried such minute worms that they were not detected at that time. In view of the unusually high incidence among Fulton fish throughout 1966–67, it might be argued that the Fulton area is more likely to be a focal point of infection than is the Sandspit–Lakehead region.

Daily Infection Incidence

The inverse relation between magnitude of daily smolt emigrations and their infection incidence, particularly toward the end of both early and late runs (see Fig. 2) is believed to occur because bursts of healthy fish are superimposed upon a more regular output of infected ones. This might be expected if infected fish have poor swimming performance as suggested by Smith and Margolis (1970) and as a result migrate more slowly, or if as Lester (1970) suggested for sticklebacks infected with *Schistocephalus*, physical distortion of infected fish caused them to school separately. Substantial numbers of smolts with high worm counts near the ends of the runs (see Fig. 4) may be "stragglers" which have been unable to keep up with the bulk of the emigrants.

Rate of migration of infected smolts is probably an important influence on cestode population size in the following year since cestodes long in transit to the outlet would seem most likely to attain the gravid state and emit eggs before entering the river. This supposes an abundance of intermediate hosts since high parasite egg production may infect few fishes of the following year-class if copepod production is low. Low abundance or aberrant timing of plankton blooms along with that of host fishes, could account for abrupt changes in incidence in successive years as noted earlier for 1953-54 and 1969-70.

Infection Intensity

Seasonal patterns of intensity of *E. salvelini* in Babine sockeye appear rather similar over the years but causes of this are still largely speculative. Nevertheless, their consideration at this time should

be helpful to continuing studies. In both 1966 and 1967, worm burdens (aggregate weights)were larger in early than in late runs and detailed observations of 1967 showed a decline from peak numbers of cestodes late in the early run to stable lower numbers in the late run and increasing burdens through May 29. It seems unlikely that decreasing intensities after May 29 are due simply to increasing numbers of Main Lake fish having had fewer opportunities for infection since incidence in Main Lake fish was double that of North Arm fish in 1967. However it could be explained if some or all large worm populations in individual fish underwent diminution of numbers, perhaps through a rather unique population control about the time of smolt migration.

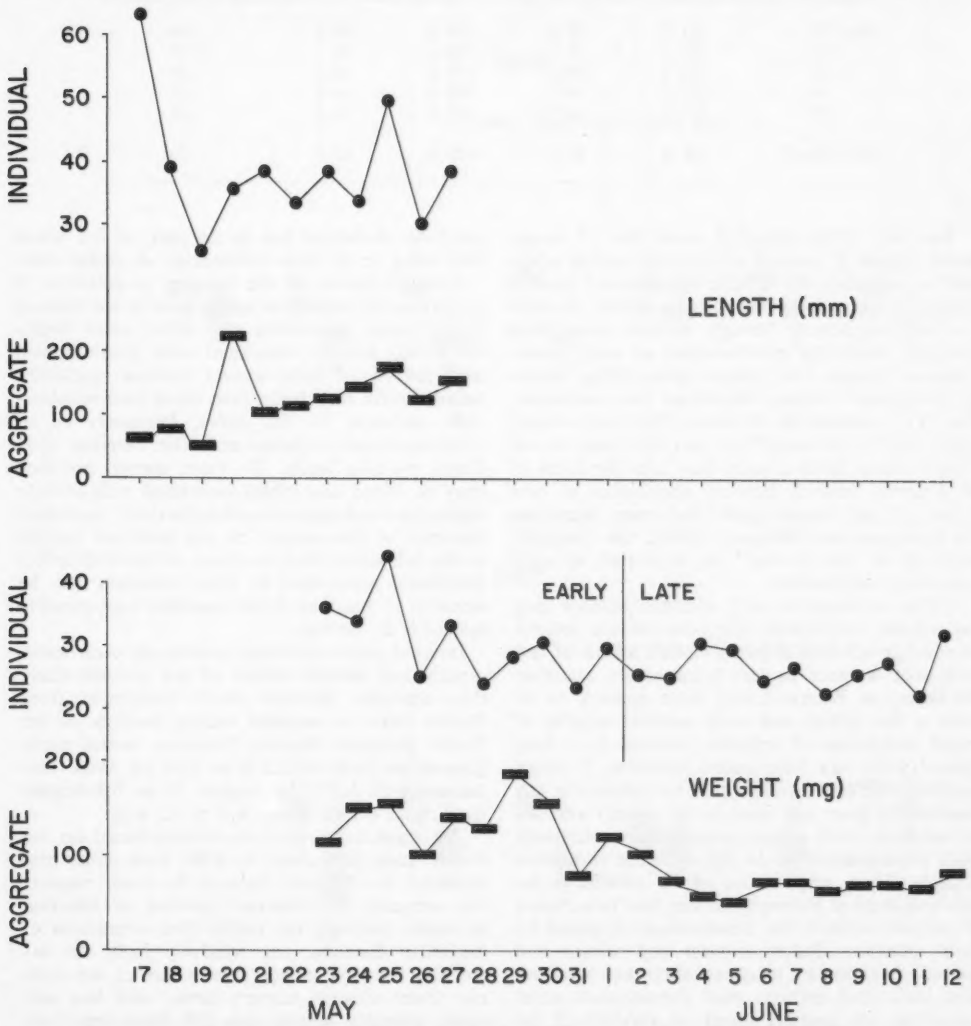

FIG. 5. Geometric mean lengths, May 17–27 and weights May 23–June 12 of cestodes in daily samples of 1967 smolt run. Upper connected points show means of individual worms; lower symbols show means of aggregate lengths and weights per fish.

TABLE 4. Geometric means of individual and aggregate lengths and weights of worms, and mean weight of worms per unit of length during a period of high incidence and intensity of infection in 1967. (Aggregates are totals of all worms in a single host.)

Date	Mean worm length (*mm*)		Mean worm wt (*mg*)		Mean wt (*mg*) per (*mm*) worm length
	(Aggr.)	(Individ.)	(Aggr.)	(Individ.)	
May 23	123.5	38.3	116.0	36.0	.94
24	146.9	33.3	150.8	34.1	1.02
25	169.9	49.6	152.1	44.4	.90
26	125.5	30.2	103.8	24.9	.82
27	167.5	38.7	143.0	33.0	.86
May 23–27	138.9	34.0	130.8	32.0	.94

Kennedy (1969) reported some loss of larger, more mature *E. crassum* of probably marine origin carried upstream by Atlantic salmon, and there is ample evidence for some such regulation in other cestode populations (though perhaps most often through restricting establishment of early developmental stages). For instance premunition, wherein established worms discourage new infections, has been reported by Roberts (1961) and others, and Chubb (1963) and Hopkins (1959) have shown that in some fishes control may take the form of a dynamic balance between acquisition of new infections and loss of established ones. According to Halvorsen and Williams (1968), the "carrying capacity of the habitat" is implicated in such regulatory mechanisms.

Either premunition or a dynamic balance may be efficient mechanisms for host–parasite systems to regulate infection intensity when a source of new infections is more or less continuously available. However, at Babine Lake, there appears to be only a few spring and early summer months of rapid acquisition of cestodes followed by a long period with very little added infection. It seems unlikely that lost worms would be replaced in late summer or later and because of normal attrition of numbers, small summer populations could under such circumstances be in risk of being eliminated entirely. Thus, perpetuation of *E. salvelini* in juvenile sockeye at Babine Lake may best be achieved if cestodes tolerate the disadvantages imposed by large numbers during autumn and winter and exercise final control in spring when their numbers and bulk (and perhaps their physiological state) constitute the greatest threat to survival of the host–parasite system.

It is possible that an immune response, as demonstrated by Kennedy and Walker (1969) could preclude reinfection late in the year, of fish which lost some or all their cestodes at an earlier date.

Little is known of the location or activities of underyearling sockeye in spring prior to ice breakup but it seems reasonable that when water begins to warm, activity associated with smoltification and pursuit of food should increase markedly. Many smolts then begin their travel over considerable distances to the outlet, frequently in an undernourished condition and often carrying maximum parasite loads. To these energy demands may be added any others associated with cestode maturation and egg production. In short, maximum demands of the cestode on the intestinal habitat in the fish seems likely to occur at this time and a downward adjustment in worm numbers may be essential at that time if the combined host–parasite system is to survive.

Infected smolts surviving to reach the ocean grow rapidly and adverse effects of the parasite likely then diminish. Infected smolts transferred from Babine Lake to seawater rearing facilities at the Pacific Biological Station, Nanaimo carried mean *Eubothrium* loads of 2.1% in June 18, 1968. This decreased to 1.2% by August 12 as fish tripled their mean weight (from 4.0 to 12.4 g).

The suggested control mechanism based on the Babine Lake data seems to differ from either premunition or dynamic balance in some respects but accounts for observed patterns of infection in smolts reaching the outlet after migrations of dissimilar duration, i.e. relatively high but decreasing intensity during later stages of the early run from adjacent nursery areas, and low and stable intensity among late fish from the more distant Main Lake.

Intensity of *E. salvelini* may be characteristically 1–2 worms per fish at the host-smolt stage as report-

ed for smolt samples for six separate Pacific coast streams in 1956 (Margolis 1963). Vik (1963) found much higher intensities in adult char infected with the parasite in Norway, however.

Effects on the Fish

There is no direct evidence that *E. salvelini* causes death of juvenile sockeye migrating and living in Babine Lake though several observations suggest it may occur. An instance of heavily infected smolts dying in holding pens at the lake outlet was cited earlier and with results of unpublished swimming performance tests of infected and noninfected Babine smolts suggests that the survival capability of infected ones may be low. Also a decline in overall infection intensity, in numbers of smolts carrying five or more worms, and changes in the

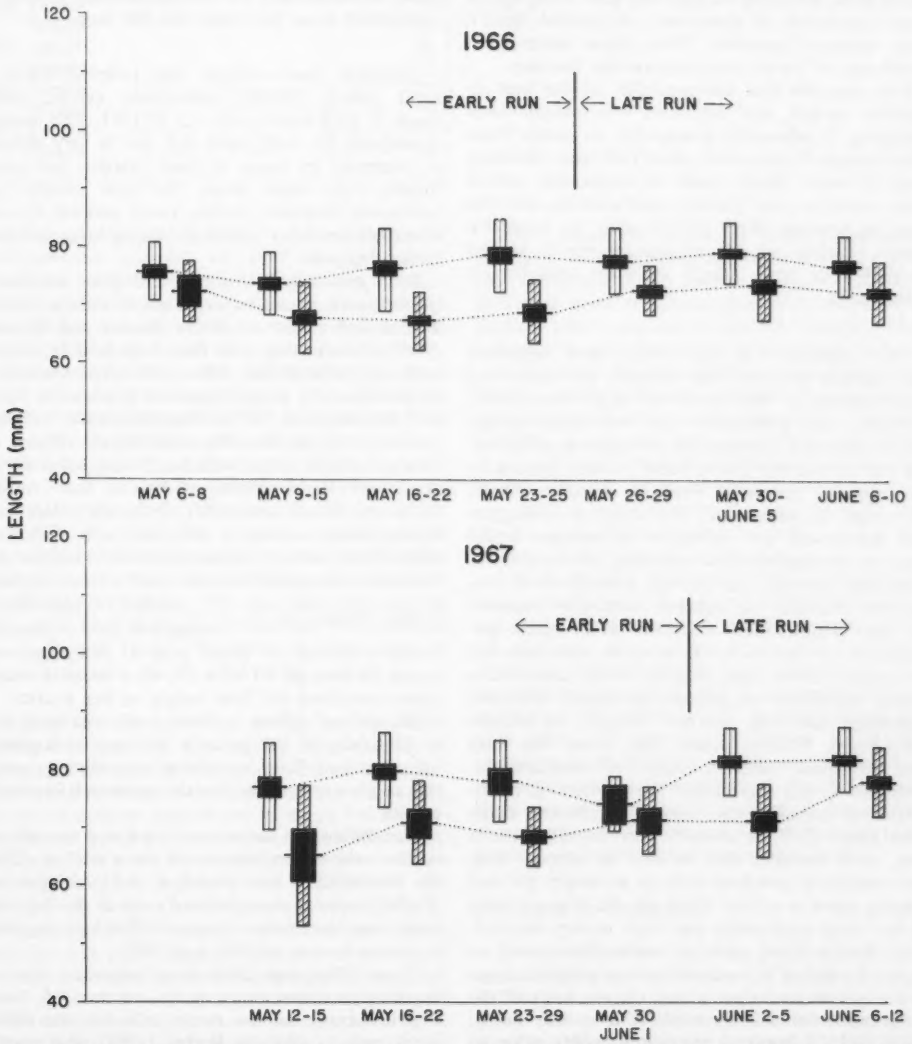

Fig. 6. Lengths of infected and noninfected sockeye smolts in sample periods of 1966 and 1967. Symbols show mean, SD and 2 SE for each sample.

mean-size relationships of infected and noninfected fish in consecutive sample periods in both 1966 and 1967 smolt runs, e.g. from May 23–29 to May 30–June 1, 1967, could have resulted from mortality among heavily infected, smaller fish.

Microscopic inspection of stained 10μ sections of pyloric caeca, each harboring 1–3 cestodes, revealed portions of host epithelium drawn into worm bothria, but no obvious tissue damage. Likewise no intestinal damage has been noted during many thousands of dissections of infected Babine Lake sockeye juveniles. Thus, there seems little likelihood of losses from mechanical damage.

It is possible that the capability of the host to perform certain vital functions was lowered thus rendering it unusually susceptible to death from other causes. In that event, poor nutrition, excessive load, or toxic effects could be implicated, and if these result in poor growth, postlacustrine survival may be lowered. Rees (1967), after an extensive review reached no definite conclusions as to the possibility of toxic effects of adult cestodes on their hosts.

Poor nutrition — It has often been suggested that mature fish are little affected by nutritional requirements of their cestodes (e.g. Rees 1967). However, such conclusions may not apply equally well to juveniles, and recently, Williams and Halvorsen (1971) asserted that a belief in the intestine as an unlimited source of food for cestodes should no longer be supported. This appears to suggest that substantial gut occlusion by cestodes could result in inadequate host nutrition, presumably by restricting passage, or through absorption of significant amounts of nutrient materials required for host maintenance. Instances of complete gut occlusion of salmon by *E. crassum* were reported by Dogiel (1964), and Wardle (1933) observed a similar condition in hatchery-produced salmonid fingerlings involving cestodes thought to include *Eubothrium*. Wardle noted that some fish that died exhibited external signs of malnutrition. Chandler (1942) has raised the interesting possibility that cestodes may absorb nutritional fluids from areas of direct contact with the host gut. If true, some cestodes may be able to support their own metabolic processes even in an empty gut and thereby cause a critical drain on the host at times of low food availability and high energy demand.

At Babine Lake, sockeye smolts often carry so large a burden of *E. salvelini* that complete blockage of the gut seems to occur and on the basis of the foregoing it could conceivably lead to their death, particularly if seaward migration occurs prior to appreciable spring zooplankton blooms. (Timing of such blooms in Babine Lake is indicated below.) Since Babine sockeye smolt–adult survival rates average only about 6% (range 2.0–9.4% for brood years 1959–66), and because an appreciable part of the mortality likely occurs prior to ocean residence, as suggested for Fraser River sockeye by Williams (1969), rather small reductions in energy reserve seem likely to put smolts at considerable disadvantage. Nutritional losses which might in other circumstances be inconsequential, cannot be considered so at this time and life stage.

Excessive load — About one infected smolt in eight (about 200,000 individuals) carried worm loads $\geqslant 10\%$ in the early run of 1967. This appears prodigious for such small fish but is very difficult to interpret in terms of their relative well-being. Smolts with loads about this size usually had noticeably distended bellies, swam awkwardly, and often attracted our attention among large numbers in holding pens.

Such physical aberrations and poor swimming performance would be expected to reduce feeding success and attract predators. Becker and Brunson (1967) observed that trout fingerlings heavily infected with *Diphyllobothrium* were particularly susceptible to predation by larger trout and piscivorous birds, and Rogers et al. (1972) observed similar selective predation by Arctic char (*Salvelinus alpinus*) on young sockeye parasitized by *Trianophorus* in the Agulowak River system of Bristol Bay, Alaska. Similarly, Pennycuick (1971) believed sticklebacks (*Gasterosteus aculeatus*) infected with *Schistocephalus* were weakened and susceptible to heavier predation than noninfected ones. She used a "parasite index" defined as $\text{PI} = \frac{\text{weight of parasites}}{\text{weight of fish} + \text{parasites}}$ which is similar to "load" used in this paper, and found an average PI of 0.27, or a parasite weight about one-third the host weight in her studies.

Size-related effects — Poor host nutrition, due to demands of the parasite or because excessive load lessened food gathering capabilities, would reduce growth rates in the lake and result in smaller smolts.

Size differences between infected and noninfected smolts were appreciable in all years studied (Table 5). Noninfected fish averaged 5–7% longer and 17–24% heavier than infected ones in the four late runs, and somewhat greater differences occurred in the early runs of 1966 and 1967.

These differences likely have important survival implications. For example, a number of North Pacific sockeye salmon stocks including the Babine stock were studied by Ricker (1962) who showed that highest sea survival was generally associated

TABLE 5. Lengths (mm), and weights (g), and differences (%) in length and weight of infected and noninfected smolts in late smolt runs of 1952 and 1953, and early, late, and total runs of 1966 and 1967. Data of 1952 and 1953 from Dombroski (1955).

Year		Early run			Late run			Both runs		
		Infected	Non-infected	Difference (%)	Infected	Non-infected	Difference (%)	Infected	Non-infected	Difference %
1952	Length				77.8	81.8	4.9			
	Weight				4.2	5.1	17.6			
1953	Length				82.3	88.4	6.9			
	Weight				5.3	6.7	20.9			
1966	Length	67.9	75.4	9.9	72.6	78.1	7.0	70.0	76.5	8.5
	Weight	2.8	4.1	31.7	3.4	4.5	24.4	3.1	4.3	27.9
1967	Length	68.1	77.0	11.6	75.7	81.4	7.0	72.2	79.9	9.6
	Weight	3.5	5.4	35.2	4.7	5.8	19.0	4.1	5.7	28.1

with largest smolts. Similarly, in current studies of postlacustrine survival of 1967 Babine smolts conducted by the author and J. McDonald, marked individuals averaging 78 mm fork length produced about twice as many adult recoveries as did those averaging 72 mm. Small size, parasitism, or both, could be chiefly responsible, but if infection *causes* small size, the parasite seems at best an indirect reason for mortality in the 1967 run since the bulk of the 72 mm fish were infected (see Fig. 5).

While it appears resonable to postulate a causative rather than associative relationship between fish size and *Eubothrium* infection, the latter cannot be entirely dismissed. For instance, early migrating fry might at times obtain some growth advantage and encounter fewer infected copepods; later fish might never overcome the size difference and at the same time devour an abundance of infected food. Dogiel (1964), however, believes that *E. crassum* retards growth of Atlantic salmon.

Effects of other parasites on size — Dombroski (1955) first reported that smolts infected with *E. salvelini* were smaller than noninfected ones, but that the latter were in turn smaller than about 12% of smolts without *Eubothrium* infection but harboring the nematode *Philonema oncorhynchi* (Kuitenen-Ekbaum 1933). *Philonema*-infected underyearlings from Babine Lake in the autumn of 1959 were also found to be larger than nonparasitized ones by L. Margolis and N. P. Boyce (personal communication), and this was noted again during current studies. It is not known whether the size-related effects resulted from differential feeding opportunities (a postulate of Dombroski 1955) or because, as suggested by L. Margolis (personal communication), the nematode may in some way stimulate host growth.

There seems no reason to doubt Dombroski's assertion that "apparent effects on growth is probably solely due to the cestode infection." However, studies of possible interrelated effects of these and perhaps other parasites on Babine sockeye production may be a useful line of investigation in future.

In summary, the extent of loss of juvenile sockeye salmon attributable to infection by *Eubothrium salvelini* is at this point highly speculative, but some, perhaps largely indirect mortality undoubtedly occurs. If population control is primarily exercised at the time of increased host activity in spring, any cestode-induced mortality among smolt year-classes might be maximized by weak, or late functioning of the control mechanism.

Clearly, there is need to show the relative weights of population control and host mortality in shaping seasonal changes in both incidence and intensity of infection. Also needed are studies to measure effects of infection upon young salmon at several levels of intensity. Difficulties in interpreting field observations of "surviving fish" and correlating them with effects of parasitism at earlier life stages is apparent. Such problems may be partially overcome through better understanding of the life cycle and dynamics of infection. Investigation into both of these matters is in progress and enough is now known to erect an hypothesis to explain the latter.

DYNAMICS OF INFECTION

Several million smolts, many infected with ovigerous cestodes, pass through the greater length of Babine Lake each spring and seem likely to be a prime source of infection for the following salmon year-class. If true, this egg source along with known seasonal abundance of cyclopoid copepods and

other plankters, and changing food preferences of young fish, suggest a sequence of infection consistent with the timing and behavior of young sockeye and accounting for the midsummer stabilization of underyearling infection incidence.

Zooplankton composition and abundance were measured at many stations and depths during May–October, 1952–62 (Johnson 1964) and dry weights by species, depth, and station are available (Johnson 1965). Figure 7 shows that cyclopoid copepods are commonest in early spring (their maximum biomass also frequently occurs then) but decline to a seasonal low in July. Cladocerans, by contrast, are in low abundance in spring but increase rapidly in late July or August. A similar succession was observed by Narver (1970) in plankton of the North Arm where, despite an abundance, juvenile sockeye had stopped eating copepods by August, in favor of other plankters, notably cladocera. Such a shift in eating habits in midsummer would clearly minimize new infections thereafter and produce a stable infection incidence

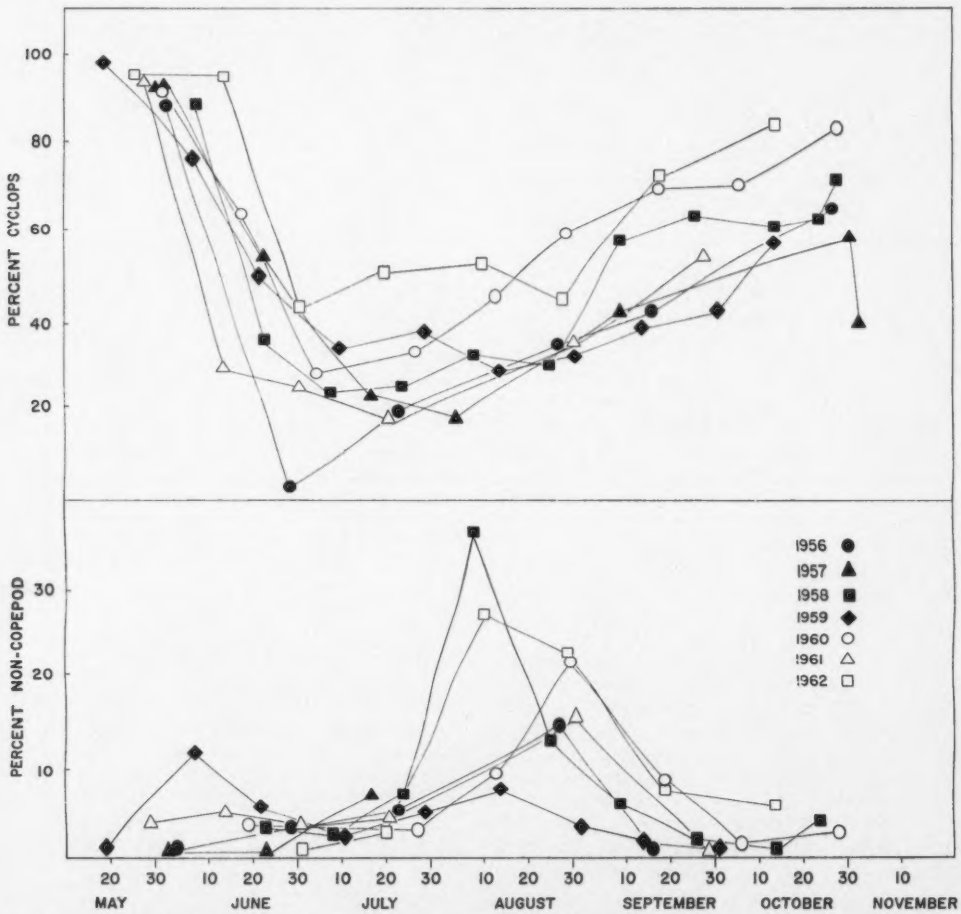

FIG. 7. Relative abundance of *Cyclops scutifer* and *C. bicuspidatus* (*upper*), and of noncopepods, largely *Bosmina* sp. and *Daphnia* sp. (*lower*) in zooplankton near Bolings Pt., 1956–62. Nauplii and early copepodid stages were omitted to avoid possible errors from misidentification, and calanoid copepods (*Diaptomus* spp.), which were an abundant food item but otherwise not implicated in the hypothesis were included only in calculations of percent noncopepods. Figures compiled from data of Johnson (1965).

in under yearlings in August–October. The south-north arrival of spring with corresponding warming of the lake and evacuation of smolts would ensure stabilization of infection levels in the same order.

Two other factors could be instrumental in terminating new infections after midsummer. First, eggs produced in spring could by summer have sunk to the bottom or below easy access to copepods, or they could die naturally. Second, if there are two generations of *Cyclops* per year at Babine — a first in May and June with a life span of 3–4 months, and a second in September with a span of 8–12 months — as suggested by K. Patalas (personal communication), individuals of the spring generation would likely be too small to ingest cestode eggs, and late copepodid and adult stages which did ingest them could have reached the end of their life span by August.

These several factors suggest that if smolts are the principal source of parasite eggs, acquisition of cestodes by underyearlings is likely to diminish substantially after midsummer and the pattern of uptake of infection shown in Fig. 6 may be expected. The extent of cestode egg production in other fishes must be evaluated as a test of one element of the hypothesis.

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AWACHIE, J. B. E. 1966. Observations on *Cyathocephalus truncatus* Pallas, 1971 (Cestoda: Spathebothriidae) in its intermediate and definitive hosts in a trout stream, North Wales. J. Helminthol. 40: 11.

BANGHAM, R. V., AND J. R. ADAMS. 1954. A survey of the parasites of freshwater fishes from the mainland of British Columbia. J. Fish. Res. Board Can. 11: 673–708.

BECKER, C. D., AND W. D. BRUNSON. 1967. *Diphyllobothrium* (Cestoda) infections in salmonids from three Washington Lakes. J. Wildlife Manage. 31: 813–824.

BYKHOVSKAYA-PAVLOVSKAYA, I. E., ET AL. 1964. Key to the parasites of freshwater fishes of the USSR, No. 80. (Transl. from Russian by Israel Program for Sci. Transl., Jerusalem, No. 1136: 919 p.)

CHANDLER, A. 1942. Studies on the nutrition of tapeworms. Amer. J. Hyg. 37: 121–130.

CHUBB, J. C. 1963. Seasonal occurrence and maturation of *Triaenophorus nodulosus* (Pallas, 1781) (Cestoda, Pseudophyllidea) in the pike, *Esox lucius* L., of Llyn Tegid. Parasitology 53: 419–433.

DOGIEL, V. A. 1958. Ecology of the parasites of freshwater fishes. *In* Parasitology of fishes. Oliver & Boyd, Edinburgh. (Transl. from Russian by Z. Kabata.)

1964. General parasitology. Oliver & Boyd, Edinburgh and London. 516 p. (Transl. from Russian by Z. Kabata.)

DOMBROSKI, E. 1955. Cestode and nematode infection of sockeye smolts from Babine Lake, British Columbia. J. Fish. Res. Board Can. 12: 93–96.

FOERSTER, R. E. 1968. The sockeye salmon *Oncorhynchus nerka*. Fish. Res. Board Can. Bull. 162: 422 p.

GUNNERØD, T. B. 1971. The life history and population dynamics of *Cyclops scutifer* Sars in Pedro Bay, Iliamna Lake, Alaska. Ph.D. Thesis. Univ. of Washington, Seattle, Washington.

GUTTOWA, A. 1963. Natural focus of infection of plankton crustaceans with procercoids of *Diphyllobothrium latum* L. in Finland. Acta. Parasitol. Pol. XI: 145–153.

HALVORSEN, O., AND H. H. WILLIAMS. 1968. Studies on the helminth fauna of Norway. IX. *Gyrocotyle* (Platyhelminthes) in *Chimaera monstrosa* from Oslo Fjord, with emphasis on its mode of attachment and a regulation in the degree of infection. Nytt Mag. Zool. 15: 130–142.

HOFFMAN, G. L. 1967. Parasites of North American freshwater fishes. Univ. California Press, Berkeley and Los Angeles, Calif. 486 p.

HOPKINS, C. A. 1959. Seasonal variation in the incidence and development of the cestode *Proteocephalus filicollis* (Rud. 1810) in *Gasterosteus aculeatus* (L. 1766). Parasitology 49: 529–542.

JOHNSON, W. E. 1956. On the distribution of young sockeye salmon (*Oncorhynchus nerka*) in Babine and Nilkitkwa Lakes, B. C. J. Fish. Res. Board Can. 13: 695–708.

1964. Quantitative aspects of the pelagic, entomostracan zooplankton of a multibasin lake system over a 6-year period. Verh. Int. Ver. Limnol. 15: 727–734.

1965. Quantitative studies of the pelagic entomostracan zooplankton of Babine Lake and Nilkitkwa Lake, 1955–1963: Methods, stations and basic data. J. Fish. Res. Board Can. MS Rep. 821: 224 p.

JOHNSON, W. E., AND C. GROOT. 1963. Observations on the migration of young sockeye salmon (*Oncorhynchus nerka*) through a large, complex lake system. J. Fish. Res. Board Can. 20: 919–938.

KENNEDY, C. R. 1969. The occurrence of *Eubothrium crassum* (Cestoda: Pseudophyllidea) in salmon *Salmo salar* and trout *S. trutta* of the River Exe. J. Zool. (London) 157: 1–9.

1971. The effect of temperature upon the establishment and survival of the cestode *Caryophyllaeus laticeps* in orfe, *Leuciscus idus*. Parasitology 63: 59–66.

KENNEDY, C. R., AND P. J. WALKER. 1969. Evidence for an immune response by Dace, *Leuciscus leuciscus*, to infections by the cestode, *Caryophyllaeus laticeps*. J. Parasitol. 55: 579–582.

LESTER, R. J. G. 1970. The influence of *Schistocephalus* plerocercoids on the respiration of *Gasterosteus* and a possible effect on the behaviour of the fish. Can. J. Zool. 49: 361–366.

MACLULICH, D. A. 1943. Parasites of trout in Algonquin Provincial Park, Ontario. Can. J. Res. 21: 405–412.

MARGOLIS, L. 1963. Parasites as indicators of the geographical origin of sockeye salmon, *Oncorhynchus nerka* (Walbaum), occurring in the North Pacific Ocean and adjacent seas. Int. N. Pac. Fish. Comm. Bull. No. 11: 101–156.

MCDONALD, J. G. 1969. Distribution, growth and survival of sockeye fry (*Oncorhynchus nerka*) produced in natural and artificial stream environments. J. Fish. Res. Board Can. 26: 229–267.

NARVER, D. W. 1970. Diel vertical movements and feeding of underyearling sockeye salmon and the limnetic zooplankton in Babine Lake, British Columbia. J. Fish. Res. Board Can. 27: 281–316.

PENNYCUICK, L. 1971. Seasonal variations in the parasite infections in a population of three-spined sticklebacks *Gasterosteus aculeatus* L. Parasitology 63: 373–388.

REES, G. 1967. Pathogenesis of adult cestodes. Helminth Abstr. 36: 23 p.

RICKER, W. E. 1962. Comparison of ocean growth and mortality of sockeye salmon during their last two years. J. Fish. Res. Board Can. 19: 531–560.

ROBERTS, L. S. 1961. The influence of population density on patterns and physiology of growth in *Hymenolepis diminuta* (Cestoda: Cyclophyllidea) in the definitive host. Exp. Parasitol. 11: 332–371.

ROGERS, D. E., L. GILBERTSON, AND D. EGGERS. 1972. Predation-prey relationship between Arctic char and sockeye salmon smolts at the Agulowak River, Lake Aleknagik, in 1971. Univ. Wash. Coll. Fish. Circ. 72-7: 40 p.

SMITH, H. D., AND L. MARGOLIS. 1970. Some effects of *Eubothrium salvelini* (Schrank 1970) on sockeye salmon, *Oncorhynchus nerka* (Walbaum), in Babine Lake, British Columbia. J. Parasitol. 56 (11): 321–322.

SMYTH, J. D. 1963. The biology of cestode life cycles. Tech. Commun. No. 34, Commonw. Bur. Helminthol., St. Albans, Herts, England. 38 p.

VIK, R. 1963. Studies of the helminth fauna of Norway. IV. Occurrence and distribution of *Eubothrium crassum* (Bloch 1799) and *E. salvelini* (Schrank 1790), (Cestoda) in Norway, with notes on their life cycles. Nytt Mag. Zool. 11: 47–73.

WARDLE, R. A. 1933. The parasitic helminths of Canadian animals. I. The Cestodaria and Cestoda. Can. J. Res. 8: 317–333.

WARDLE, R. A., AND J. A. MCLEOD. 1952. The zoology of tapeworms. Univ. Minnesota Press, Minneapolis. 780 p.

WILLIAMS, H. H., AND O. HALVORSEN. 1971. The incidence and degree of infection of *Gadus morhua* L., 1758 with *Abothrium gadi* Beneden, 1871 (Cestoda, Pseudophyllidea). Norw. J. Zool. 19: 193–199.

WILLIAMS, I. 1969. Implication of water quality and salinity in the survival of Fraser River sockeye smolts. Int. Pac. Salmon Fish. Comm. Progr. Rep. 22: 46 p.

YAMAGUTI, S. 1959. Systema helminthum. Vol. II. The cestodes of vertebrates. Interscience Publishers Inc., New York and London. 860 p.

Effect of Thermal Shock on Vulnerability of Juvenile Salmonids to Predation[1]

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COUTANT, C. C. 1973. Effect of thermal shock on vulnerability of juvenile salmonids to predation. J. Fish. Res. Board Can. 30: 965-973.

Thermally shocked juvenile rainbow trout and chinook salmon were selectively preyed upon by larger trout in the laboratory when exposure times to elevated temperatures exceeded a minimum duration. This duration was 10% (chinook) and 20% (rainbow) of the exposure duration that caused obvious loss of equilibrium (complete body inversion) of half a test population at that temperature. Longer exposures increased vulnerability to predation relative to controls almost exponentially. Shorter exposures made shocked fish less susceptible to predation. Shocked fish showed some recovery from heat effects when returned to the initial holding temperature for ½ or 1 hr prior to predation. Susceptibility of rainbows to predation (relative to controls) appears to follow the time- and temperature-dependant response pattern previously shown by others for death and visible equilibrium loss. Selective predation began at exposures about 10% of the median death time in the range 26 to 30 C.

COUTANT, C. C. 1973. Effect of thermal shock on vulnerability of juvenile salmonids to predation. J. Fish. Res. Board Can. 30: 965-973.

De jeunes truites arc-en-ciel et des saumons chinook soumis à un choc thermique deviennent sélectivement la proie de truites plus grandes lorsque la durée d'exposition aux températures élevées dépasse un certain minimum. Cette durée est de 10% (saumon chinook) et de 20% (truite arc-en-ciel) de la durée qui provoque une perte évidente d'équilibre (renversement complet du corps) de la moitié d'une population d'essai soumise à cette température. Des expositions plus longues augmentent presque exponentiellement la vulnérabilité à la prédation comparativement à celle des témoins. Les expositions plus courtes rendent les poissons moins susceptibles à la prédation. Les poissons se remettent quelque peu du choc lorsqu'on rétablit la température initiale de maintenance pendant une demi-heure ou une heure avant de les exposer à la prédation. La susceptibilité des truites arc-en-ciel à la prédation, comparativement à celle des témoins, semble se conformer au type de réponse liée au temps et à la température que montrent d'autres poissons vis-à-vis la mort ou la perte visible d'équilibre. La vulnérabilité sélective à la prédation commence à des expositions d'environ 10% du temps léthal moyen, dans l'intervalle de 26 à 30 C.

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THIS study sought to determine whether sublethal exposure of young salmonids to potentially lethal high temperatures would measurably increase their vulnerability to predation by larger fish. This question arose because the tested species, juvenile chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Salmo gairdneri*), received thermal shocks of various magnitudes in passing through mixing zones of thermal discharges from the Hanford plutonium production reactors near Richland, Washington during their seaward emigration from the upper Columbia River (Becker et al. 1971).

The possibility of active predation upon thermally shocked young fish was suggested by several observations: (1) young fish in laboratory thermal resistance time experiments (similar to those of Brett 1952) exhibited a characteristic loss of equilibrium at some time prior to cessation of opercular beating, which is generally accepted as the endpoint for physiological death (Coutant and Dean 1972). This equilibrium loss occurred at exposure times averaging as low as 40% of the lethal exposure duration for some temperatures; (2) predatory fishes and gulls occurred in large numbers near the thermal mixing zone of at least one of the Hanford

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reactors (Prentice 1969); (3) predation by larger fishes is important in affecting survival of juvenile salmonids under natural conditions (Ricker 1941; Thompson 1959; Roos 1960).

Two series of experiments are reported herein. The first tested several durations of exposure to one potentially lethal high temperature for each of the two prey species. The objectives of this series were to determine if differential predation could be identified quantitatively, and if so, how the perception of stress by actual predators compared with loss of equilibrium as identified by an investigator. The other series examined the response of rainbow trout to three high temperatures. It sought to determine if increased susceptibility to predation follows a classical "dose response" in which the effect is a function of shock temperature and duration of exposure, and the time required to produce the effect is inversely proportional to the temperature.

Materials and Methods

Each test was conducted according to the flow diagram in Fig. 1. Juvenile fish were first removed from a common stock tank and both shocked and control groups were marked with a liquid nitrogen cold brand (Fujihara and Nakatani 1967). After a minimum of 72 hr for recovery from handling and for appearance of a strong brand, 25 individuals of each group were transferred to exposure tanks, one containing heated water, the other containing water at the holding (acclimation) temperature. Thermal exposure tanks were supplied with flowing water that had been air-equilibrated after heating to avoid additional stress from gas supersaturation.

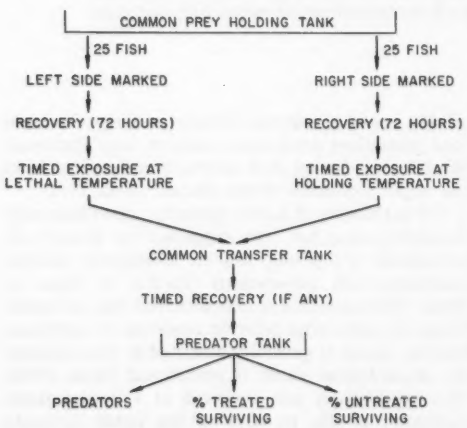

FIG. 1. Flow diagram for conduct of tests to determine the relative vulnerability of thermally shocked and control fish to predation.

Temperatures were maintained by a control valve that mixed hot and cold water supplies.

On completion of the timed thermal exposure, shocked and control groups were reunited at the holding temperature in a 9-liter plastic pail for transfer to the predator tank. The fish were gently stirred to ensure mingling of the two groups. In some tests, fish were then held together at the initial holding temperature for a timed period to test for possible recovery from heat effects. Experimental recovery periods lasted ½ or 1 hr. In tests without recovery time, the mixed groups were offered to the predators within 1 min, by dumping the entire contents of the transfer pail into the predator tank.

Predation took place in 4- or 6-ft diameter (1.2 or 1.8 m) circular fiberglass tanks with water depths of 65 and 80 cm, respectively. A continuous exchange of water was maintained at about 15 liters/min entering at the perimeter surface and leaving via a double standpipe from the center bottom. There were no structures other than inlet pipe, outflow standpipe, and tank walls to provide visual protection to the prey. Although Mossman (1955) called attention to the theoretical effectiveness of walls as cover (by limiting the angles of attack and necessitating "braking" by the predator to avoid collision), it was impossible to conduct these tests without some tank structures.

The predators used in all of these tests were large rainbow trout. Large rainbow are common in the central Columbia River, both as residents and during migrations of the anadromous "steelhead" form. They were voracious predators and ate well in the laboratory. Initial tests used adults more than 3 years old from our laboratory brook stock. Two-year-olds, which were more agile in the test tanks, were used for most tests. There were three to five predators per tank. There was no indication that predators increased their selectivity for a given group of prey after several tests, so they were used repeatedly.

After introduction of the prey, predators were allowed a maximum of ½ hr to remove approximately 50% (about 25 fish), at which point predation was stopped by rapid removal of the survivors. Shocked and control fish were then counted. In some tests, length and weight determinations were also made. A predation period longer than ½ hr was undesirable because recovery of shocked fish would not be accounted for. Established predators generally consumed the required number of fish within 3–10 min. Surviving prey were not used in subsequent trials as a precaution against learning or long-term effects of thermal shock.

Bams (1967) discussed the reasons for limiting the numbers of prey consumed to one-half of those added. The principal reason for standardizing the percentage eaten is the changing ratio of prey availability during a test, i.e. the decrease in relative abundance of the preferred group. The measured rate of predation on the preferred group, based on results of the overall test, is lower than the actual instantaneous rate for that group. This discrepancy increases as predation proceeds. Standardizing the number of prey eaten reduces variability in this factor. In practice, this procedure was difficult to adhere to, because active feeding precluded keeping accurate count of remaining prey. Tests were not con-

sidered valid if more than 35 or fewer than 15 of the initial 50 prey remained. The results for a particular test therefore include a bias proportional to the indicated rates of predation and the difference between the number of all fish remaining and 25. Determination of this bias is not easily made and was not carried out.

The statistic chosen by Bams (1967) and used in this study to express the difference in predation rates upon the two groups of fish is the ratio $d_p = \frac{i_1}{i_2}$, where i_1 and i_2 are, respectively, the instantaneous mortality rates of the first (thermally shocked) and the second (control) group. The instantaneous mortality rate when time is a unit interval is given by $i = -\log_e s$, where $s =$ survival proportion, i.e. $\frac{\text{No. at finish}}{\text{No. at start}}$. The chi-square analysis was used to test for homogeneity among trial results and for significance of observed differences in predation rates.

Because performance of fish varies greatly with size, length was analyzed as a variable that could affect rates of predation in this study, even though all fish were small (as detailed below). Lengths of survivors from some tests were measured and compared with equal numbers of stock fish measured at periodic intervals. Lengths of survivors in thermally shocked and control groups from each experiment were tested for significant differences using Student's *t*-test.

The initial series of experiments conducted in 1968 and 1969 tested different durations of exposure to one potentially lethal shock temperature for each prey species. The shock temperatures were 28.0 ± 0.05 C for chinook and 30.5 ± 0.05 C for rainbow. These temperatures are within the lethal ranges for the test species. Median times to death at these temperatures were about 22.5 and 15.5 min, respectively. Exposures were administered that were fractions of the median exposure duration that causes loss of equilibrium, since the initial objective was to relate differential predation rates to equilibrium loss observed by an investigator.

Juvenile chinook salmon for these tests were raised in the laboratory from eggs secured from adults captured at Priest Rapids Dam, 43 km upstream. The fish averaged 64.3 mm (SD = 9.9) from snout to hypural plate and 3.8 g (SD = 1.9) when tests were run in July and August of 1968 and 1969. All were held at 15 C. Juvenile rainbow were raised in the laboratory from adult brood stock maintained there for several years. Rainbow averaged 75.4 mm (SD = 9.6) and 8.0 g (SD = 2.7). Rainbow were not held at a controlled constant temperature as were chinook, but were held in water pumped directly from the Columbia River at temperatures that varied from about 17 to 19 C just prior to and during the tests.

In the second series of experiments in the fall of 1969, rainbow trout were tested at three temperatures, 26, 28, and 30 C each with a series of exposure durations that identified the exposure time which initiated an increased susceptibility to predation. These rainbow were raised in the laboratory from the same brood stock used previously. The fish averaged 95.03 mm (SD = 15.74) and 16.26 g (SD = 6.97). They were held at 15 C for several weeks before testing. Because the main stock of young fish had reached a larger average size when these experiments were run in the fall, the stock was divided, and only the smallest fish were used. This nonrandom selection for size occurred prior to separation of fish for shocked and control groups, and is believed to have no significance for the results.

To compare various endpoints of thermal stress, two other endpoints (median death time and median time to loss of equilibrium) were determined for the stock of rainbow used in the 1969 tests. Death times were determined in the manner described by Brett (1952); equilibrium loss was determined as described by Coutant and Dean (1972).

Single Shock Temperature

In tests where rainbow trout were offered as prey immediately after thermal exposure, the predators captured significantly more thermally shocked fish ($P < 0.05$) after exposure durations amounting to 30, 40, and 50% of the median time for loss of equilibrium (Table 1, Fig. 2). There was no statistically significant selective predation on thermally shocked fish at exposures of 15, 20, and 25%, although a trend toward selection of shocked fish at the longer exposures is evident. Thermally shocked fish actually showed better survival at 15% than did the controls.

The exposure duration which would first cause an increase in average relative vulnerability to predation is estimated from Fig. 2 to be about 18% of the median time for loss of equilibrium, or about 90 sec. This cannot be a precise estimate, however, since there was considerable variability in the results of replicate tests.

There was some recovery when rainbow were held for a half hour at the initial holding temperature before being offered to the predators (Table 1, Fig. 2). Statistically significant selection of shocked fish occurred after thermal exposures amounting to 80 and 100% of the median time for loss of equilibrium, but not after 50 or 60%. Results of replicates at 50% were not homogeneous. The exposure time required to first cause an increased susceptibility to predation after ½-hr recovery appears to be near 50% of the median equilibrium loss time.

Exploratory tests with young chinook salmon in 1968 indicated that this species was also more vulnerable to predation after thermal exposures shorter than those producing median loss of equilibrium (Table 2). More extensive tests in 1969 showed that exposure times of 10, 20, 30, and 50% of the median time for equilibrium loss caused significantly ($P < 0.01$) greater predation on thermally shocked fish (Table 2, Fig. 3). Selection was not significantly different ($P < 0.05$) after exposures lasting 5% of the equilibrium loss time.

TABLE 1. Average numbers of survivors (from 25 unless noted) and ratios (combined for replicates) of instantaneous predation rates (d_p) for 1968 tests with juvenile rainbow trout fed to adult rainbow after different thermal exposure times (holding temp. = 17–19 C, shock temp. = 30.5 C) [$d_p = \frac{i \text{ shocked}}{i \text{ control}}$, where $i = -\log_e$ (survival proportion)].

Duration of exposure % of EL_{50}[a]	sec	No. of replicates	Avg no. surviving shocked	control	Combined chi square (1 df)	Heterogeneity X^2	Combined d_p
			No recovery				
15	75	3	16.0	12.0	0.857	1.56	0.608
20	100	8	14.5	15.4	0.205	4.79	1.120
25	125	2	7.5	13.5	1.714	2.21	1.954
30	150	3	11.7	20.0	6.579	0.23	3.412
40	200	4	12.8	18.8	4.571	1.87	2.340
50	250	5	3.4	17.8	48.905	5.08	4.873
			½-hr recovery				
50	250	4	12.0[b]	13.0	0.061	9.25	1.075
60	300	2	14.0[b]	20.0	0.866	1.61	2.025
80	400	4	10.5[b]	20.0	11.457	5.67	3.842
100	500	3	11.0[b]	18.7	3.914	1.49	2.474

[a] EL_{50} = Median time to loss of equilibrium.
[b] Some tests had fewer than 25 fish at the start.

There is a clear trend of rising ratios of instantaneous predation rates with increasing length of exposure time (Fig. 3). An exposure duration which would first cause selective predation on thermally shocked young chinook appears to be very near 5% (33 sec) of the median time for equilibrium loss.

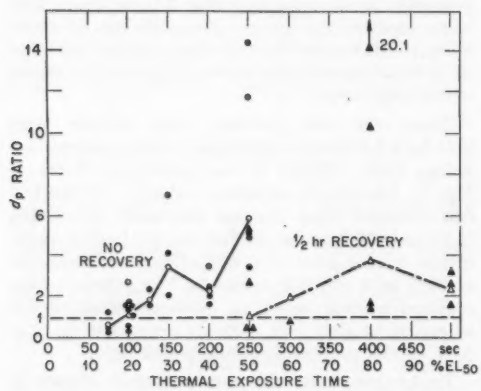

FIG. 2. Increasing ratios of instantaneous predation rates (d_p ratio) for juvenile rainbow trout with increasing thermal exposure times (shown in seconds and in % of median time for loss of equilibrium), for predation immediately following thermal exposure (circles) and after ½-hr recovery (triangles). Solid characters indicate individual tests, open characters indicate combined replicates. A d_p ratio of 1 (dashed line) signifies equal susceptibility of shocked and control fish to predation; $d_p > 1$ signifies greater susceptibility of shocked fish.

A holding period of ½ hr at the initial 15 C allowed the chinook to recover to an extent similar to the rainbow (Table 2, Fig. 3). The 80% exposure time yielded statistically significant differences, but 20, 40, and 50% of the median equilibrium loss time did not. The ratios at 40 and 50% were both higher than unity, however, and an intercept with unity did not occur until about the 20% level. Further experiments with a holding period of 1 hr showed that selective predation still occurred at the 80% exposure time.

Surviving shocked and control prey were never significantly different in length. Both were different from stock fish in some tests, however. Of 15 tests for which length data were analyzed in detail, five tests had survivors with significantly different lengths than the stock. The direction of the difference was not consistent; the survivors were larger than the stock in three experiments but smaller in the other two.

THREE SHOCK TEMPERATURES

The three shock temperatures, 26, 28, and 30 C, induced selective predation of young rainbow over different ranges of exposure times. The highest

temperature exhibited effects after the shortest exposures (Table 3, Fig. 4).

The lowest test temperature, 26 C, induced significantly different predation after exposures of 64, 90, and 120 min, but not after 16 or 32 min. The trend of rising d_p ratios with increasing exposure duration (Fig. 4) suggests that an exposure of about 32 min would first induce changes in prey behavior or performance that would result in an increased vulnerability of shocked fish to predation. Fish exposed for 16 min actually showed less susceptibility to predation than did the controls.

At the highest shock temperature of 30 C, a 1-min exposure yielded a group chi-square (3.373) that was only slightly below the 0.05 level of significance. The difference in predation was highly significant for exposure times of 1.5 min and more. The trend in d_p ratios (Fig. 4) suggests that an exposure time of 0.55 min or more would induce selective predation on shocked fish.

The supply of young rainbow was exhausted before the lowest exposure time resulting in increased predation at 28 C could be determined. Tests with exposures lasting 4, 8, and 16 min yielded highly significant differences in predation, however. An extrapolation of these data (dotted line in Fig. 4) suggests that increased predation on shocked fish may first occur at exposures near 2 min.

The three combinations of temperature and exposure time that were estimated to first induce selective predation on thermally shocked young rainbow exhibited a pattern of time dependancy similar in form to the data obtained for equilibrium loss and death (Fig. 5). When end points for predation and death are compared on the time scale, selective predation occurred at an average exposure time that was 10.9% of the median death time for that temperature. Comparison along the temperature scale indicates that increased vulnerability to predation occurred at temperatures about 2.5 C lower than for death at the same exposure time.

Discussion

This study has demonstrated that thermal stress which is considerably less stringent than is necessary for death or equilibrium loss visible to an investigator can increase the vulnerability of young salmon

TABLE 2. Average numbers of survivors (from 25 unless noted) and ratios (combined for replicates) of instantaneous predation rates (d_p) for tests with juvenile chinook salmon fed to adult rainbow trout after different thermal exposure times (holding temp. = 15 C, shock temp. = 28 C) (calculated as in Table 1).

Duration of exposure % of EL_{50}[a]	sec	No. of replicates	Avg. no. surviving: shocked	Avg. no. surviving: control	Combined chi square (1 df)	Heterogeneity X^2	Combined d_p
			1968 Exploratory tests, no recovery				
50	357	4	6.0	19.0	27.040	8.91	5.200
100	714	3	2.7	20.0	39.764	1.81	10.029
			1969 Tests, no recovery				
5	33	7	10.4	12.0	0.770	1.96	1.191
10	67	6	9.5	19.0	19.000	6.96	3.525
20	134	4	6.0	21.5	34.945	1.75	9.462
30	201	4	1.8	22.5	71.020	0.37	25.239
50	335	2	1.0	24.0	42.320	0.00	78.851
			1969 Tests, ½-hr recovery				
20	134	6	11.8	13.5	0.657	0.97	1.213
40	268	4	8.5[b]	13.0	3.588	0.30	1.634
50	335	4	10.3[b]	14.5	2.267	1.63	1.561
80	536	4	7.8[b]	17.5	13.898	2.36	3.198
			1969 Tests, 1-hr recovery				
80	536	4	7.8	15.3	5.820	4.38	2.064

[a] EL_{50} = Median time to loss of equilibrium.
[b] Some tests had fewer than 25 fish at the start.

and trout to predation. The reappears to be a minimum duration of exposure necessary at a given test temperature to induce increased predation on the shocked fish. Beyond this exposure time, vulnerability relative to controls increases almost exponentially. Curiously, shorter exposure durations often made the shocked fish less susceptible than the controls, perhaps due to nervous stimulation or increase in locomotory functions by an increase in body temperature.

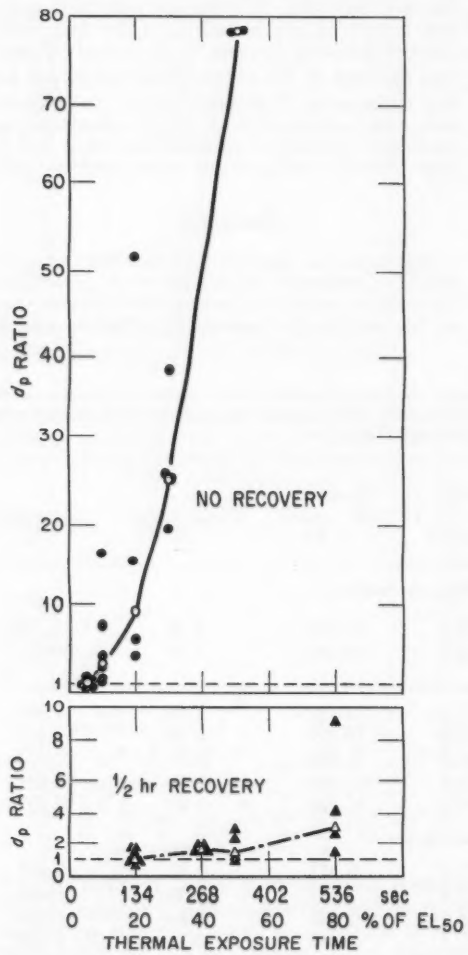

FIG. 3. Increasing ratios of instantaneous predation rates (d_p ratio) for juvenile chinook salmon with increasing exposure times, for predation immediately following thermal exposure (*upper*) and after ½-hr recovery (*lower*). Notation as in Fig. 2.

Increase in susceptibility to predation immediately after thermal shock appears to follow the time- and temperature-dependant response pattern previously shown by Fry et al. (1946) and Brett (1952) to be exhibited by the death response, and by Coutant and Dean (1972) to be exhibited by equilibrium loss visible to an investigator. When graphed in the manner introduced by Fry et al. (1946), the three response patterns appear as roughly parallel lines. These relationships suggest the suitability of using computational "correction factors" to estimate the conditions of temperature and time that cause differential predation on other prey species when median death times for them are available. The factors would be 1) reduction of the exposure time at a given temperature to about 10%, or 2) reduction of the exposure temperature by about 2 C for a given exposure time.

The stressing effects of brief thermal shocks that affect predation appear to be reversible to some degree with holding time in cool water. A ½-hr holding period before predation lengthened by three or four times the exposure time necessary for occurrence of statistically significant differences in predation.

The recovery data suggest that there may have been two levels of thermal damage, or that there are two recovery mechanisms. The shortest durations of thermal exposure that induced selective predation when there was no postshock holding did not clearly induce selective predation following a ½-hr recovery. The components of performance affected by these short exposures must, therefore, return quickly to normal. However, fish given longer thermal exposures remained more susceptible to predation (although to a much reduced extent), even after 1 hr was allowed for recovery. This may imply a more permanent thermal damage. Additional study using longer recovery times will be necessary to clarify these responses.

Thermal shock may affect many of the behavioral and performance factors that allow a prey fish to avoid predation. Bams (1967) discussed these factors in a general way, but he was not concerned with thermal stress. These factors include discovery, attack, capture, retention, availability, level of predation, and relative sizes of predators and prey. Discovery, attack, and capture seem to be most affected by thermal shock in these experiments.

Behavior of the shocked fish presumably increased the rate at which predators discovered them and hence the rate of predation. When given a severe thermal exposure equal to the median time to loss of equilibrium, half of the shocked fish were render-

ed unable to swim upright. Although most regained equilibrium on return to cooler water, their behavior remained abnormal. They exhibited disorientation, erratic bursts of swimming activity, and often an unnatural posture (head at the surface with the tail angled downward). Such patterns probably caused rapid discovery by the predators. In fact, obviously disoriented prey were among the first to be eaten. The predators may have discerned similar, but subtler, changes in behavior (e.g. the flashing side of a distressed fish) even after thermal exposures much shorter than needed for obvious equilibrium loss.

After discovery of the prey, the predator may have attacked test and control groups at different rates, i.e. exerted selection by accepting or rejecting each potential prey on the basis of some prey characteristic. McPhail (1969) observed such discrimination in the attack of two races of threespined stickleback (*Gasterosteus aculeatus*) by the western mudminnow (*Novumbra hubbsi*) in laboratory tests where predatory activity was slow and easily observed.

Abnormal prey behavior patterns probably varied in degree from long to short thermal exposures, and provided more suitable cues for initiating predator pursuit than the behavior of unshocked fish. Mossman (1955) cites evidence that suggests provocation of attacks by sign stimuli associated with weakness of the prey species. R. A. Bams (personal communication) suggested that attack rate may be more effective than enhanced discovery in laboratory tests where the predator can see many prey simultaneously and choose among them.

Poor escape performance by shocked fish may have made them more susceptible to capture by predators. Escape performance is probably a complex interaction of several prey abilities, including detection of pursuit, burst swimming capacity, agility in changing swimming direction, and capability for a prolonged series of extreme exertions. None of these could be measured independently in these tests. However, fish that received long sublethal thermal exposures were obviously less responsive to stimulation with a forceps than were unshocked fish from the same source. This change in responsiveness may have reduced their ability to recognize predators and to take evasive action. Shocked fish were often slow to seek shelter in the predation tanks however futile this attempt was. Decreased swimming ability of starved, diseased, or otherwise weakened bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) was identified by Herting and Witt (1967) as the probable cause of an increased predation by bowfin (*Amia calva*).

TABLE 3. Average number of survivors (from 25) and ratios (combined for replicates) of instantaneous predation rates (d_p) for juvenile trout fed to adult rainbow after several exposure times each to 26, 28, and 30 C (holding temp. = 15 C). Calculations are as in Table 1.

Duration of exposure (*min*)	No. of replicates	Avg no. surviving		Combined $\underline{X^2}$ (1 df)	Heterogeneity $\underline{X^2}$	Combined d_p
		shocked	control			
		26 C Shock temp.				
16.0	4	15.8	12.3	1.750	1.61	0.647
32.0	2	15.0	16.0	0.065	0.09	1.144
64.0	2	9.5	18.0	5.254	0.15	2.945
90.0	3	8.0	21.7	18.887	1.10	7.962
120.0	2	4.5	23.5	25.785	0.80	27.713
		28 C Shock temp.				
4.0	4	5.8	20.5	33.152	6.24	7.405
8.0	3	6.0	21.0	25.000	4.94	8.185
16.0	3	5.0	23.0	34.714	0.71	19.302
		30 C Shock temp.				
0.5	4	14.8	14.3	0.034	0.60	0.938
1.0	4	11.0	15.8	3.373	2.89	1.776
1.5	6	6.7	16.5	25.043	1.57	3.181
4.0	4	5.8	20.5	33.152	6.24	7.405
8.0	3	6.0	21.0	25.000	4.94	8.185
16.0	3	5.0	23.0	34.714	0.71	19.302

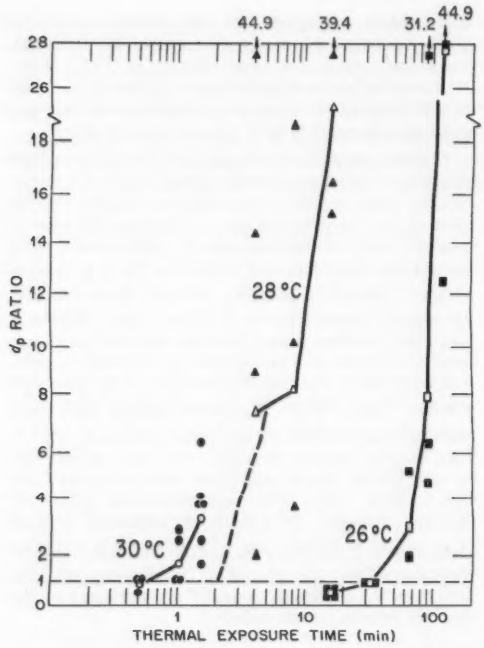

FIG. 4. Time dependance of three patterns of ratios of instantaneous predation rates (d_p ratio) for juvenile rainbow trout tested at three temperatures. Notation as in Fig. 2.

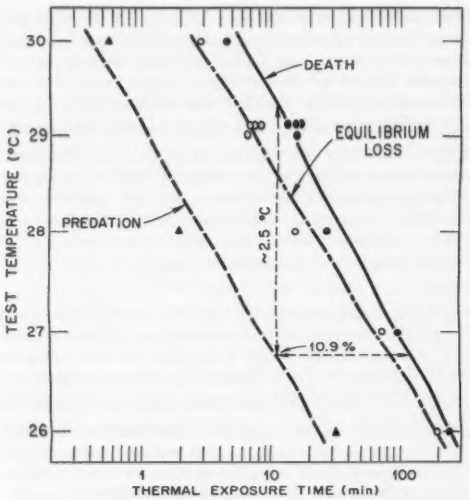

FIG. 5. Relationships among three effects of acute thermal shock on a sibling group of juvenile rainbow trout acclimated to 15 C: (1) time to initial increase in vulnerability to predation, (2) median time to loss of equilibrium, and (3) median death time.

Length differences among prey seem to have played a minimal role in these experiments. Because shocked and unshocked prey were initially from the same stock groups, length differences were small compared to the differences examined by Bams (1967) and others who concluded that length was very important for swimming performance.

These tests simulated, in simplified manner, the conditions believed to exist near the Hanford thermal discharges where the principal thermal shocks were received only by the prey. Here, juvenile salmonids passed with strong river currents through the zones of maximum temperatures (that exceeded 48 C) while predators waited outside this zone in slightly elevated temperatures (2–3 C) to which they were capable of acclimating metabolically. The principal simplification was exposing the fish to a constant high temperature, rather than the fluctuating shock temperatures that occurred in the river (Becker et al. 1971; Jaske et al, 1970). In an actual thermal plume, fish received an abrupt temperature rise followed by a fluctuating, gradual temperature decrease to a mixed temperature slightly above the initial temperature.

Experimental fish were actually subjected to two thermal shocks. The first was an abrupt rise in temperature at the time of transfer to the heated water. The second was an abrupt decrease of similar amount on return to the holding temperature. The latter shock induced momentary loss of equilibrium in some fish not showing these symptoms in the heated bath. The independent effects of these two shocks could not be assessed in the experiments, although it is appreciated that "cold shock" may also be important in its own right since it occurred ust prior to predation.

Despite these simplifications, the predation experiments reported here strongly suggest that thermal discharges can be evaluated for environmental impact in terms of their potential for inducing selective predation on valuable prey species. The conditions of elevated temperature and duration of exposure that would be stressing to exposed prey would be much less than those suggested by analysis of thermal death data. The differences in predation rates in these experiments provide a quantitative measure of harmful effect. Therefore the data should be suitable for developing scientific design criteria for thermal releases which will not be stressing to aquatic organisms.

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BAMS, R. A. 1967. Differences in performance of naturally and artificially propagated sockeye salmon migrant fry, as measured with swimming and predation tests. J. Fish. Res. Board Can. 24: 1117–1153.

BECKER, C. D., C. C. COUTANT, AND E. F. PRENTICE. 1971. Experimental drifts of juvenile salmonids through effluent discharges at Hanford. Part II. 1969 drifts and conclusions. U.S. At. Energy Comm. Res. Develop. Rep. BNWL 1527: 61 p.

BRETT, J. R. 1952. Temperature tolerance in young Pacific salmon, genus *Oncorhynchus*. J. Fish. Res. Board Can. 9: 265–323.

COUTANT, C. C., AND J. M. DEAN. 1972. Relationships between equilibrium loss and death as responses of juvenile chinook salmon and rainbow trout to acute thermal shock. U.S. At. Energy Comm. Res. Develop. Rep. BNWL 1520: 19 p.

COWLES, R. B., AND C. M. BOGERT. 1944. A preliminary study of the thermal requirements of desert reptiles. Bull. Amer. Muss. Natur. Hist. 83: 265–296.

FRY, F. E. J., J. S. HART, AND K. F. WALKER. 1946. Lethal temperature relations for a sample of young speckled trout (*Salvelinus fontinalis*). Univ. Toronto Stud., Biol. Ser. No. 54, Publ. Ont. Fish. Res. Lab. No. 66: 5–35.

FUJIHARA, M. P., AND R. E. NAKATANI. 1967. Cold and mild heat marking of fishes. Progr. Fish. Cult. 29: 172–174.

HERTING, G. E., AND W. WITT, JR. 1967. The role of physical fitness of forage fishes in relation to their vulnerability to predation by bowfin (*Amia calva*). Trans. Amer. Fish. Soc. 96: 427–430.

JASKE, R. T., AND M. O. SYNOGROUND. 1970. Effect of Hanford Plant operations on the temperature of the Columbia River 1964 to present. U.S. At. Energy Comm. Res. Develop. Rep. BNWL 1345: 378 p.

JASKE, R. T., W. L. TEMPLETON, AND C. C. COUTANT. 1970. Methods for evaluating effects of transient conditions in heavily loaded and extensively regulated streams. Proc. 1969 ASME/AICHE Stream Pollut. Abatement Conf., Chem. Eng. Progr. Symp. Ser. 67: 31–39.

MAINS, E. M., AND J. M. SMITH. 1964. The distribution, size, time, and current preferences of seaward migrant chinook salmon in the Columbia and Snake Rivers. Fish. Res. Pap., Wash. Dep. Fish. 2: 5–43.

MCPHAIL, J. D. 1969. Predation and evolution of a stickleback (*Gasterosteus*). J. Fish. Res. Board Can. 26: 3183–3208.

MOSSMAN, A. S. 1955. Experimental studies of fitness as measured by vulnerability to predation. Ph.D. Thesis. Univ. Wisconsin, Madison, Wis. 134 p.

PARK, D. L. 1969. Seasonal changes in downstream migration of age-group O chinook salmon in the upper Columbia River. Trans. Amer. Fish. Soc. 98: 315–317.

PRENTICE, E. F. 1969. Gull predation in a reactor discharge plume. U.S. At. Energy Comm. Res. Develop. Rep. BNWL 1050, Part 2: 2.33–2.34.

RICKER, W. E. 1941. The consumption of young sockeye by predacious fish. J. Fish. Res. Board Can. 5: 293–313.

ROOS, J. F. 1960. Predation of young coho salmon on sockeye salmon fry at Chignik, Alaska. Trans. Amer. Fish. Soc. 89: 377–379.

THOMPSON, R. B. 1959. Food of the squawfish *Ptychocheilus oregonensis* (Richardson) of the lower Columbia River. U.S. Fish Wildl. Serv. Fish. Bull. 158: 43–58.

Thermal Requirements for Maturation, Spawning, and Embryo Survival of the Brook Trout, *Salvelinus fontinalis*

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Thermal requirements for brook trout reproduction were determined at a natural day-length cycle and five constant temperatures (10–21 C) and one treatment that followed the seasonal temperature regime of Lake Superior. The optimum range for growth (unrestricted rations) and relative condition factor was 10–19 C. Survival was 90% or greater and gonad growth and secondary sexual characteristics were well developed at all temperatures. The maximum temperature at which males became functionally mature and contained motile spermatozoa was 19 C. Ovulation and spawning occurred at 16 C and lower. Water temperature had little influence on time of spawning, but had a major influence on spawning activity and egg viability. The upper median effective temperature (ET50) for the number of viable eggs spawned per female was 11.7 C. The upper median tolerance limit (TL50) for normal hatch was 12.7 C; the optimum temperature was near 6 C. Major factors influencing the number of embryos surviving to hatch were spawning and incubation temperatures and age of the embryo when exposed to test temperatures. Thermal criteria that recognize seasonal changes in their thermal requirements are recommended for self-sustaining brook trout populations.

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Les auteurs ont déterminé les exigences thermiques de la reproduction de l'omble de fontaine dans des conditions naturelles de photopériode et à cinq températures constantes (10–21 C), en plus d'un traitement qui a suivi le régime saisonnier de températures du lac Supérieur. La gamme optimale pour la croissance (rations illimitées) et l'indice relatif d'embonpoint est de 10–19 C. Le pourcentage de survie est de 90 ou plus et le développement des gonades et des caractères sexuels secondaires se poursuit normalement à toutes les températures. La température maximale à laquelle les mâles deviennent effectivement matures et possèdent des spermatozoïdes doués de mouvement est de 19 C. L'ovulation et la fraie ont lieu à 16 C ou moins. La température de l'eau n'a que peu d'influence sur le déclenchement de la fraie, mais affecte grandement l'activité reproductrice et la survie des oeufs. La température supérieure effective médiane (ET50) du nombre d'oeufs viables pondus par femelle est de 11.7 C. La limite supérieure de tolérance médiane (TL50) d'une éclosion normale est de 12.7 C; l'optimum est près de 6 C. Les principaux facteurs influençant le nombre d'embryons qui survivent jusqu'à l'éclosion sont la température au moment de la fraie et durant l'incubation et l'âge de l'embryon au moment où il est soumis aux températures expérimentales. On devrait tenir compte des changements saisonniers d'exigences thermiques dans l'établissement de critères de température pour des populations autonomes d'omble de fontaine.

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THE threat of excessive waste heat from the growing electric-generating industry has prompted research into the thermal requirements of important aquatic species. Early studies of fish reproduction have been concerned chiefly with the role of the photoperiod, temperature, or both on the onset of breeding behavior or functional maturity. Atz (1957) pointed out that most studies were conducted under limited conditions that were too brief to assess properly the effects of light and temperature, and stressed the need for long term experiments covering the reproductive cycle. Brungs (1971)

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exposed *Pimephales promelas* for one generation to constant elevated temperatures; spawning success decreased with increasing temperature above 23 C, and no spawning took place above 30 C.

Previous studies on the brook trout, *Salvelinus fontinalis*, have produced considerable information on their thermal requirements (Fry 1951) and life history (Bridges and Mullan 1958). Embody (1934) determined the relation of temperature to the incubation period of eggs of four species of trout. Henderson (1963) described the influence of light and temperature on the reproductive cycle of the brook trout. Her data suggests that the maturation cycle can be modified by changes in day length. These studies have practical value to the fish culturist, but they provide limited information for predicting the impact of waste heat on fish reproduction in nature under normal seasonal changes in the photoperiod.

The study reported here is part of the continuing program at the National Water Quality Laboratory to determine thermal requirements of important freshwater fish species. The purpose was to evaluate the effects of various constant temperatures on brook trout maturation, spawning, and embryo survival to hatch. Brook trout were reared and spawned naturally in laboratory tanks under a normal seasonal day-length cycle during 1968. Experiments were conducted to determine factors influencing embryo survival to hatch. This study also provides an opportunity to evaluate the experimental approach by relating the numerous field observations, experimental studies, and fish-cultural knowledge available on brook trout to our findings on reproduction in this species.

Materials and Methods

Experimental Tanks

Six fiberglass tanks with a center divider (each duplicate 150 × 27 × 35 cm deep) were used in the maturation and spawning experiment. A standpipe at the downstream end maintained water depth at 25 cm for a total volume of 100 liters in each tank. Tank positions were arranged systematically from high to low temperatures. Spawning tanks were surrounded by a black plastic curtain to shield fish from extraneous laboratory activity. Each tank was also divided into three sections by black plastic partitions which permitted free movement of fish and reduced fighting among spawning males. Spawning boxes (40 × 25 × 10 cm deep) were filled to a depth of 8 cm with graded gravel (0.5–1.0 cm) for a spawning substrate. Fluorescent lighting was controlled by a timer switch adjusted weekly to follow the seasonal day-length cycle at Duluth, Minn.

A glass incubation chamber (25 × 15 × 10 cm deep) was located above the upstream section of each spawning tank for viability checks of each spawning and one embryo survival experiment (spawning and incubation temperature). Water flowed from a manifold through capillary tubes which regulated flow through each incubation jar at 100 ml/min (mean velocity 0.14 cm/sec). An incubation jar (90-ml) was painted black and had a stainless steel screen (8 meshes per cm) cemented to its bottom. The last two embryo-survival experiments were conducted in the apparatus described in detail by McCormick and Syrett (1970).

Water Supply

Initially (until November 1, 1968) the water supply consisted of dechlorinated municipal water from Lake Superior. The mean level of total residual chloramines was 0.007 (range <0.001–0.019 μg/l) determined from weekly grab samples. During the remainder of the study, raw water was obtained directly from Lake Superior and used without treatment. This water supply (Hokanson et al. 1973) flowed through polyvinyl chloride (PVC) pipes and valves into a stainless steel headbox, before flowing into each duplicate tank at 1 liter/min.

The mean dissolved oxygen concentration was maintained at 80% (range 45–102%) air saturation at each temperature determined from weekly grab samples. When the dissolved oxygen concentrations fell below 60% air saturation in some tanks on November 18, 1968, flow rates were increased to 2 liters/min to each tank. The dissolved oxygen concentrations were maintained near air saturation in embryo survival experiments (range 94–105%).

Temperature Control

A thermoregulator controlled a solenoid valve that regulated the flow of hot or cold water through a heat-exchanger coil immersed in each headbox. Nominal temperatures selected for the maturation and spawning experiment were 10, 13, 16, 19, and 21 C, and a treatment that followed the seasonal temperature regime of Lake Superior surface water (Table 1). Nominal test temperatures for the embryo-survival experiments were 6–18 C at 3 C intervals. Thermographs were used to monitor water temperature. Their accuracy was checked daily with a mercury thermometer to the nearest 0.1 C. The 95% confidence limit of the mean temperature was usually less than ±0.1 C except during the maturation period at 10 and 13 C.

Experimental Fish

The fish for the maturation and spawning experiment were yearling brook trout obtained from the Cedar Island Hatchery, Wis., on June 25, 1968. These trout were held in outdoor ponds under natural light conditions. They were held in the laboratory at 9 C with seasonal day lengths for 3 weeks before starting the exposure. Brook trout were fed an excess ration of Oregon Moist[1] pellets once daily, supplemented with a formula-

[1]Mention of trade name does not constitute endorsement.

TABLE 1. Nominal and measured temperatures (C) recorded in brook trout reproduction and embryo survival experiments.

Nominal	Measured	
	Mean ± 95% CL	Range[a]
Maturation period (Aug. 7–Sept. 30)		
Lake Superior	11.3±0.56	7.2–15.0
10	10.8±0.25	8.7–13.2
13	13.6±0.18	12.1–14.8
16	16.1±0.07	15.6–29.0[b]
19	18.9±0.07	18.4–19.8
21	20.9±0.10	19.2–21.9
Spawning period (Oct. 1–Dec. 10)		
Lake Superior	8.2±0.50	5.0–13.7
10	10.4±0.14	7.9–14.6
13	13.2±0.08	12.5–14.8
16	16.1±0.06	15.2–16.7
19	18.9±0.09	17.0–19.5
21	20.9±0.09	19.8–22.2
Embryo survival		
6	6.2±0.14	4.2–11.5
9	9.0±0.09	8.7–14.5
12	11.8±0.11	8.1–15.2
15	15.0±0.07	14.5–16.2
18	17.6±0.15	13.5–20.7

[a]Determined from recording thermographs. Loss of temperature control was usually for less than 2 hr.
[b]Temperature rose gradually to 29 C over a 30-min period on September 7, 1968. Fish fed readily the same day, and no mortalities occurred.

tion containing 60% ground beef liver, 30% beef spleen, 8% trout starter meal, and 2% salt (Davis 1965) once a week. All tanks were cleaned daily. Prophylactic treatments were applied periodically during the maturation period. All trout were treated alike for 45 min with either formalin (1:4,000) or Roccal[1] (1:50,000).

Eggs for two embryo survival experiments (incubation temperature; incubation temperature, tempering method, age) were obtained from mature brook trout collected from the Cascade River, Cook County, Minn., on October 28, 1968. The fish were placed in a holding tank at 8 C. The eggs from five pairs were fertilized without water the next day in a constant temperature room at 6.5–8.4 C. Eggs during incubation were shielded from direct light by black plastic covers.

Survival and Growth

Ten trout were distributed randomly to each spawning tank on July 15, 1968, since secondary sexual characteristics were not well developed at this time. A randomly selected subsample of 20 fish weighed an average of 82.3 g (range 60.6–115.6 g). Temperatures were increased 2–3 C per week until the assigned test temperatures were reached.

All fish were accidently killed in both tanks at 13 C and in one tank (B) at 21 C on August 16, 1968. These fish were replaced with similar-sized fish from the same hatchery source on August 19, 1968, and were tempered to test temperatures at a rate of 2–3 C per day.

Total length, total weight (gonad plus body weight), gonad weight, and sex were recorded for each survivor on December 10, 1968. Females containing fewer than 50 large eggs at termination were recorded as spent. The length–weight relation was calculated to estimate the relative condition factor (Kn) of each fish after LeCren (1951). The logarithmic transformation of body weight and the square root transformation of the maturity index were made to meet assumptions for the analysis of variance of treatment differences (Steel and Torrie 1960).

Spawning

On August 29, 1968, one spawning box was placed in the downstream section of each tank. A second spawning box was placed in the middle section of each tank on October 15. Redd preparation was inspected twice a day. Spawning boxes suspected of containing eggs were removed and placed in a pan containing water at test temperature. A clean spawning box was immediately inserted in its place to minimize disruption of courtship behavior. A subsample of 100 eggs or less (all eggs in smaller spawnings) was carefully removed and placed in the incubation jar at its spawning temperature. Excess eggs were removed from the gravel for total counts by the sugar-flotation method of Anderson (1964).

The total number of eggs, viable eggs, and spawnings were recorded for each exposure tank. "Viability" for each egg deposition was determined as the percentage of the eggs in the subsample that developed a neural keel. The number of viable eggs was determined by summation of the products of the percentage viable eggs in the subsample and the total number of eggs in each spawning. A "spawning" is defined in this paper as the deposition of at least one viable ovum. The median effective temperature (ET50) is an estimate of the median sublethal response and was derived by plotting the number of viable eggs spawned per female, expressed as a percentage of the highest response, against temperature. The ET50 was estimated from a linear interpolation between two points bracketing the 50% level from an arithmetic plot.

Maturation

All fish were sacrificed on December 10, 1968, 2 weeks after the last spawning. Two males and two females from each 19 and 21 C treatment were also sacrificed during the spawning period (November 7).

The maturity index (gonad weight × 100/total weight) was derived for each fish after Vladykov (1956). Gonads from randomly selected specimens at each temperature were removed and fixed in 10% buffered-neutral formalin or Bouin's fluid. Tissues were processed by standard

paraffin or wet celloidin methods and sectioned at 6 and 10 μ. The sections were stained with hematoxylin and eosin or Masson's trichrome stain. The terminology of maturation stages used to describe gonadal development follows Henderson (1962, 1963).

Extra trout were retained in a 450-liter stock tank receiving 2 liters/min flow at Lake Superior temperatures. On August 5, 26, and September 23, 1968, four or five brook trout were selected randomly from the stock tank and placed in 45-liter aquaria at 21 C for 1 week. They were acclimated to test temperatures at the rate of 3 C/day. After the exposure period, trout were fin-clipped and returned to the stock tank. The surviving fin-clipped fish and eight fish retained at Lake Superior temperatures were killed on October 8. Body and gonad weights were recorded. Observations were made on sperm motility (430×) as an index to functional maturity of males. On October 10, 10 functionally mature trout were given a similar thermal treatment, and sperm motility was observed on October 18. A spawning box and two males and two females were returned to the stock tank for observations on spawning behavior of these treated fish.

Embryo Survival Experiments

Incubating eggs were attended daily throughout development. Dead eggs were removed to prevent fungus infestation. The numbers of normal, dead, and abnormal yolk-sac larvae were recorded upon hatching. Common abnormalities included spinal curvatures and edema (Fig. 1).

The two measured responses used for the embryo survival experiments were total hatch and normal (total less abnormal and dead larvae) hatch expressed as percentages of the total number of eggs. The arcsin $\sqrt{\text{percentage}}$ transformation was used for analysis of variance test of treatment differences (Steel and Torrie 1960). Three endpoints used in this paper to describe brook trout responses to temperature; optimum, optimum range, and median tolerance limit (TL50); and the distinction between egg and embryo were used, as defined by Hokanson et al. (1973).

Spawning and incubation temperatures — Subsamples of 100 eggs apiece from each of four spawnings at Lake Superior temperatures (mean 10.7 C), 10.4, and 13.2 C and each of two spawnings at 16.1 C were assigned randomly to three incubation temperatures (10.2, 13.1, and 16.2 C). The embryos were less than 24-hr old when transferred to the incubation jars used for viability checks.

Incubation temperature — Trout eggs were incubated at temperatures lower than 9 C to determine the optimum temperature for normal hatch before estimation of the median response. Subsamples of about 100 eggs from the Cascade River stock were randomly assigned to each treatment. Eggs were tempered to incubation temperature (6–18 C at 3 C intervals) at the rate of 3 C/hr.

Incubation temperature, tempering method, age — Subsamples of about 100 eggs from the Cascade River stock were randomly assigned to each treatment. Eggs were reared at 7.0 C until they were transferred to assigned incubation temperatures at water hardening, cleavage (4-celled stage, $\frac{3}{4}$-day old), gastrula (blastoderm $\frac{2}{3}$ over yolk, 18-days old), and eyed stage (44-days old). At these times they were either transferred directly or tempered (3 C/hr) to 9, 12, and 15 C. No replication was attempted because there were 24 treatments. The second-order-interaction mean square was used for estimates of error variance (Cochran and Cox 1964).

Thermal Requirements

Survival and Growth

Brook trout survival was 90% or higher at all temperatures during the spawning and maturation experiment (Table 2). Mortality occurred at 21 C only during the maturation period. At lower temperatures mortality occurred among males during the spawning season when prophylactic treatments were stopped; death followed secondary infection of torn fins and body abrasions inflicted by fighting.

Mean body weight (total wet weight minus gonad weight) and mean total length increased with temperature and reached maxima of 223 g and 26.7 cm, respectively, at 16 C (Table 2). Growth decreased progressively at higher temperatures. The expected body weight of each experimental fish was derived from solutions of their combined length–weight relationship

$$\text{Log}_{10}\, Y = -2.7933 + 3.5982\, \text{Log}_{10}\, X$$

where Y = expected body weight in grams and X = total length in centimeters.

The observations were classified into a 2 × 2 × 6 factorial design where the factors were sex, tank position, and temperature. A general linear hypothesis computer program BMD06V (Dixon 1970) was used for analysis of variance for unequal numbers of each sex. Sex and tank position (left-A and right-B) had no significant effect on mean body weight or total length ($P > 0.10$, F-test). Males (mean Kn = 1.0065) were heavier than females (mean Kn = 0.9938) of corresponding lengths ($P < 0.05$, F-test). Temperature had a significant effect on these parameters. Data were pooled accordingly in Table 2. The optimum range for relative condition factor, gain in body weight, and total length was 10-19 C.

Spawning

Spawning began at 10 C on September 28, 1968, and reached its peak during October and early

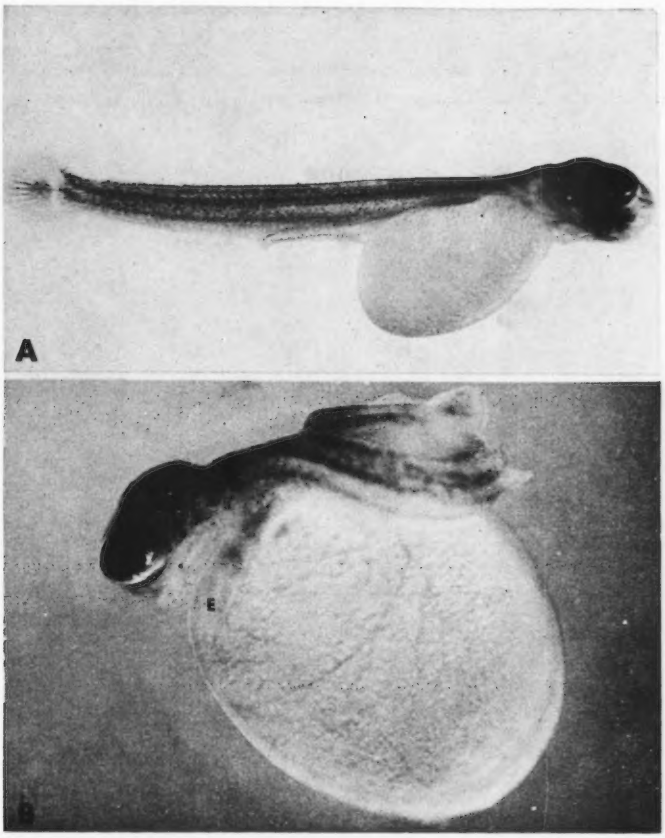

FIG. 1. Brook trout alevins: A, Normal alevin incubated at 9 C (13 mm total length); B, Deformed alevin, with edema (E) and twisted tail, incubated at 15 C.

TABLE 2. Survival, growth, relative condition, and maturity index of adult brook trout at the end of the test (December 10, 1968).

Nominal temp. (C)	Survival (%)	Mean[a] body weight (g)	Mean total length (cm)	Mean relative condition factor (Kn)		Mean[b] maturity index (%)	
				Male	Female	Male	Female
Lake Superior	90	135	23.4	1.010	0.987	0.8	1.1
10	95	174	25.2	1.003	0.996	0.4	1.0
13	90[c]	216	26.7	1.008	0.993	1.4	1.3
16	100	223	26.7	1.012	1.003	2.3	3.7
19	100	197	25.7	1.016	0.993	2.6	8.6
21	90[c]	156	24.9	0.980	0.992	1.2	16.1

[a]The logarithmic transformation of body wet weight (total minus gonad weight) was used to calculate the mean. Antilog mean body weight presented.

[b]The square root transformation of the maturity index $\frac{(\text{gonad weight} \times 100)}{\text{total weight}}$ was used to calculate the mean. Decoded mean maturity index presented.

[c]Accidental deaths occurred on August 16, and experimental chambers were restocked August 20, 1968. Mortality observed from August 20.

TABLE 3. Spawning and egg production of brook trout at six temperatures.

Nominal temp. (C) (*Duplicate*)	Sex ratio male/female	Spawnings[a]	No.[b] of eggs	No.[c] of viable eggs	Spawnings per female	Eggs per female	Viable eggs per female	No.[d] spent females
Lake Superior (*A*)	4/5	17	2857	2250	3.4	571	450	5
(*B*)	7/3	8	1567	1419	2.7	522	473	3
10 (*A*)	3/7	21	2994	1886	3.0	428	269	5
(*B*)	3/6	14	2812	2499	2.3	469	416	5
13 (*A*)	3/6	9	3001	891	1.5	500	148	3
(*B*)	5/5	7	2110	737	1.4	422	147	3
16 (*A*)	7/3	0	12	0	0	4	0	0
(*B*)	4/5	3	1038	32	0.6	208	6	3
19 (*A*)	6/4	0	0	0	0	0	0	0
(*B*)	9/1	0	0	0	0	0	0	0
21 (*A*)	5/5	0	0	0	0	0	0	0
(*B*)	4/5	0	0	0	0	0	0	0

[a]Egg depositions yielding viable eggs.
[b]Includes all infertile eggs deposited but does not include egg shells.
[c]Viability was determined when the neural keel formed. The number of viable eggs was estimated by summation of the products of the number of eggs per spawn and the percentage viable determined from a subsample of 100 or less eggs.
[d]Spent females retained less than 50 large eggs at termination.

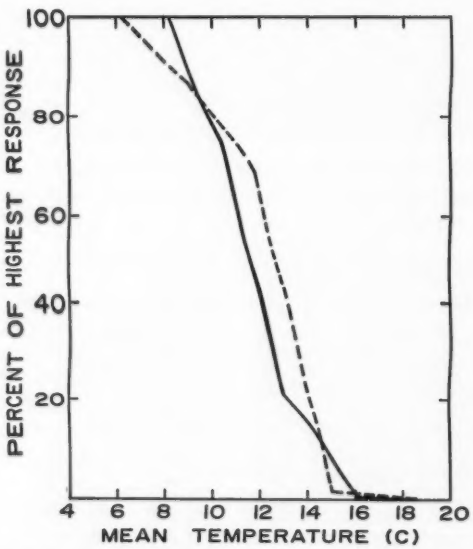

FIG. 2. Effects of temperature on brook trout reproduction — viable eggs per female (solid line) and normal hatch (dashed line). Duplicates at each treatment were pooled.

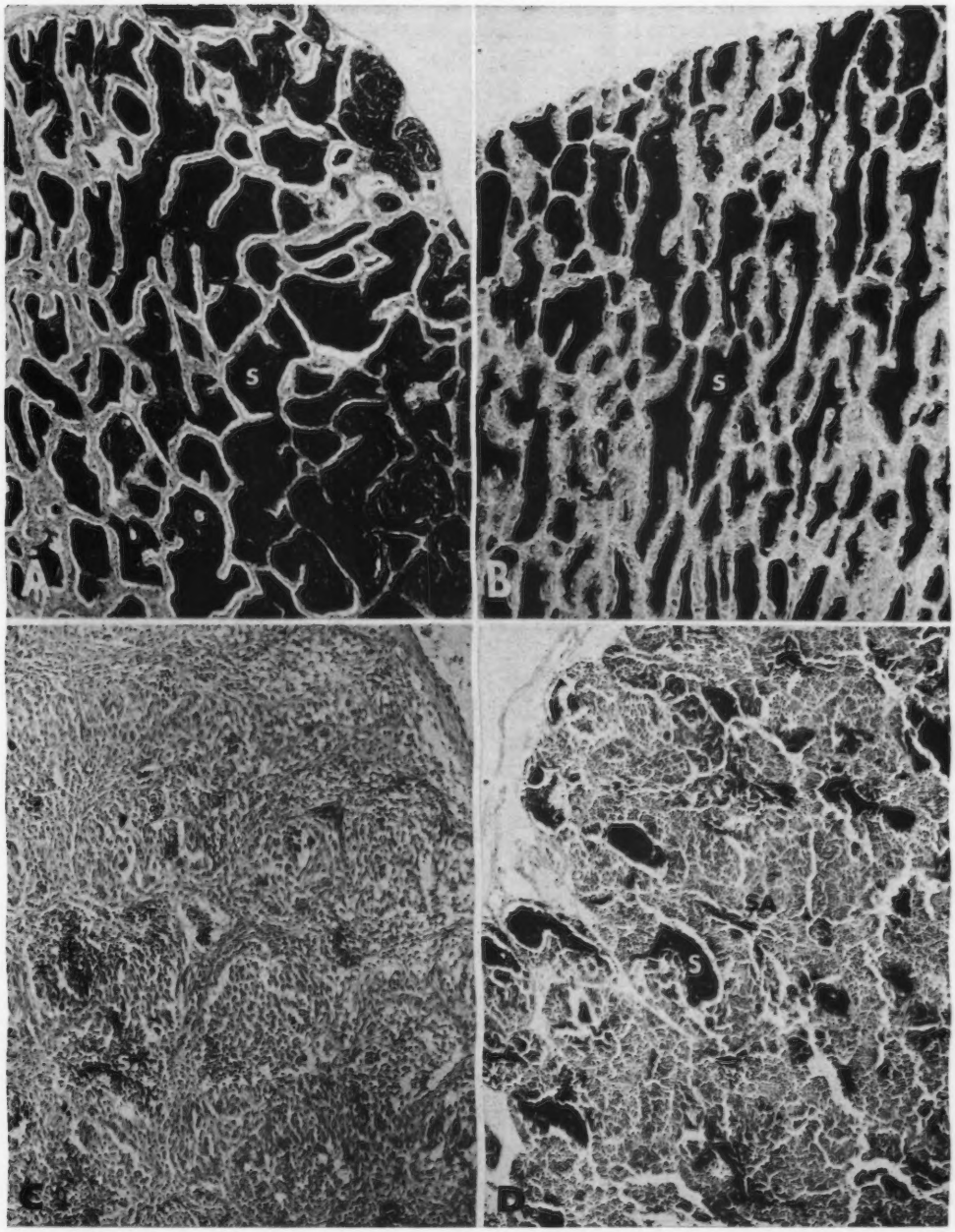

FIG. 3. Brook trout testes: A, Testis of control fish (10 C) with lobules filled with spermatozoa (S) on December 10; B, (19 C) Testis with numerous spermatozoa (S) in lobules and some spermatogenic activity (SA) still evident in periphery of lobules on November 7; C, (21 C) Testis in stage of involution with a few spermatozoa (S) still present in lobules on November 7; D, testis showing normal spermatogenic activity (SA) with some spermatozoa (S) present on July 15, before temperature elevation. Magnification A and B, 35×; C and D, 87.5×.

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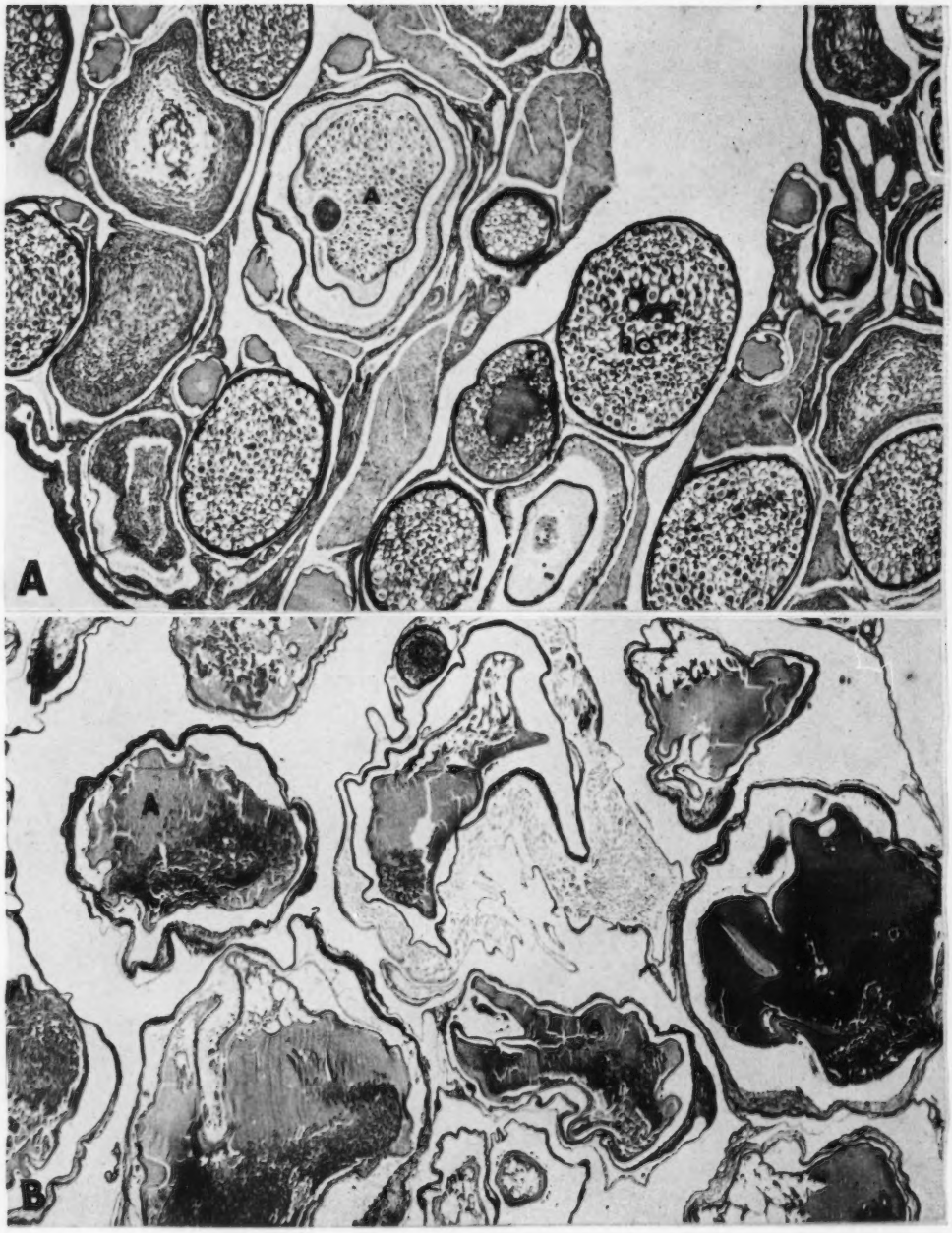

FIG. 4. Brook trout ovaries: A, (16 C) Ovary in postspawn stage with marked development of recruitment stock ova (RO) and some atresia (A) evident on December 10; B, (21 C) Ovary in early stage of resorption showing atretic ova (A) and the absence of recruitment stock ova. Magnification A, 44×; B, 18×.

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November. Spawning also occurred at and below 16 C (Table 3). Some redd-digging activity occurred in both tanks at 19 C from November 13 to 22, but no eggs were deposited. No spawning behavior was observed at 21 C. Spawning terminated on November 19, and redd-digging activity ceased after November 25 in all tanks. The number of days to spawning from September 15 followed a log-normal distribution. Mean spawning date of brook trout in all tanks was October 20, 1968 (one standard deviation included October 9 to November 4, N = 79). Mean spawning dates were October 15, 18, 24, and 28 for 10, 16, Lake Superior, and 13 C, respectively. Although analysis of variance indicated a significant difference among mean spawning dates, no relationship was evident between mean spawning date and temperature. Mean spawning date at 13 C was 13 days later than at 10 C, but no difference occurred between mean spawning dates at 10 and 16 C or between lake and 16 C ($P > 0.10$, Sheffe's test).

Fish attempted to spawn at elevated temperatures during their normal breeding season, while the number of spawnings per female, viable eggs per female, and proportion of spent females decreased progressively with increasing temperature (Table 3). Tank positions had no significant effect on the number of viable eggs per female, but water temperature did have a significant effect on this response ($P < 0.025$, F-test) (Fig. 2). The upper mean ET50 for production of viable eggs per female was 11.7 C (1 SE = 0.35 C, N= 2).

Temperature variations of the Lake Superior treatment on the day of spawning also appeared to control egg viability. Spawning began on October 9, 1968, when the preceding weekly mean temperature first fell below 13 C (range 8.0–11.6 C) since mid-August. The mean temperature during September was 13.7 C (range 12.0–15.0 C). Water temperature fluctuated daily, but decreased from 12.8 to 7.1 C during the spawning period. Egg viability of each spawning varied from 27 to 99% and averaged 82.9%. Sixty-four percent of the spawnings took place below 9.0 C. The arcsin $\sqrt{\text{percentage}}$ transformation was used for analysis of variance (Steel and Torrie 1960) and the mean response was compared at three class intervals of temperature on the day of spawning. Decoded means were 90.2% at 7.0–8.9 C, 94.4% at 9.0–10.9 C, and 70.3% at 11.0–12.9 C. Mean responses were not significantly different over the interval 7.0–10.9 C, but means at 7.0–10.9 C were significantly higher than at 11.0–12.9 C ($P < 0.05$, t-test). Daily variations in temperature below 11 C before spawning appear to enhance egg viability, since mean viability at 11.0–12.9 C was higher (70%) than expected from constant temperature exposures (ET50 for egg viability was 11.7 C).

All females held at Lake Superior temperatures were spent (Table 3). Females spawned an average of 2.7–3.4 times to deposit a total of 552–571 eggs each. Ten out of 13 females were spent at 10 C, but there were more females than males. Apparently one male can spawn two females. Spawning was incomplete at higher temperatures in spite of favorable sex ratios.

MATURATION

Effects of elevated temperatures on maturation were inferred primarily from terminal samples. These observations complement the more detailed observations of the normal reproductive cycle of brook trout described by Henderson (1962, 1963). Secondary sexual characteristics were well developed in both sexes at all temperatures. Low maturity indices at 10–16 C reflect complete and partial spawning (Table 2). Maturity indices were higher at 19 and 21 C since these fish did not spawn. The decoded-mean-maturity index (8.6) for female brook trout at 19 C was derived from only three fish (0.4, 15.6, and 17.7). On November 7 the female maturity indices were 19.7 at 19 C and 16.2 at 21 C. The mean maturity index of males at 21 C (1.2) was significantly lower than at 19 C (2.6) at the end of the test ($P < 0.01$, t-test). The mean maturity index for males at 19 C was similar to that at 16 C (2.3), where some spawning took place. For the November 7 samples, the mean maturity indices for males at 19 and 21 C were 3.4 and 2.9, respectively. The testes of trout held at 21 C were smaller than those held at 19 C ($P < 0.025$, t-test).

Male brook trout held at 10 C (N=3) were controls for histological observations (Fig. 3A). On December 10 the testes of these fish were in late stage 5 (period of functional maturity) and early stage 6 (period of involution), indicating a normal maturation cycle. The testes of two fish held at 16 C showed some indication of spermatogenic activity in the peripheral lobules, but otherwise appeared similar to those of the control fish. At 19 C (N = 2) in November, spermatozoa filled the central portion of the lobules, and spermatogenic activity was evident at the periphery (Fig. 3B). Morphologically the significant effect on the testicular cycle was seen in the four fish held at 21 C. These fish showed a marked regression of the testes and few mature spermatozoa (Fig. 3C) compared to two fish examined in July before elevation of the temperature (Fig. 3D). These changes reflect the marked differences in maturity indices between the November and December

samples at 19 and 21 C. Samples were not collected frequently enough to determine histologically if development of spermatids and spermatozoa was inhibited at 21 C. On October 10, 1968, none of four males examined at 21 C was functionally mature nor were they on November 7; however, two of three males at 19 C were functionally mature and contained motile spermatozoa on October 10 and November 7.

Short exposures of male trout to 21 C do not appear to cause permanent damage to the rapid phases of spermatogenesis (stages 3 and 4). Male trout exposed to elevated temperatures for one week beginning August 5, 26, or September 23, 1968, were all functionally mature on October 8, and all contained motile spermatozoa. Of five functionally mature males exposed to 21 C beginning October 10, only two contained motile spermatozoa a week later. By December these two males failed to spawn with two females receiving a similar treatment, though they had been returned to Lake Superior temperatures on October 17. These results suggest that spermatozoa are eliminated rapidly at 21 C if the fish are functionally mature.

Female brook trout held at 10 C showed normal maturation of the ovaries. The ovaries were in a post spawning condition in December with the recruitment stock ova (diameter < 1 mm) well advanced in number and size. A greater number of mature ova were retained in the ovary in trout held at 16 C to December than in those held at 10 C, and some atretic ova were present. Recruitment stock ova were present in these ovaries (Fig. 4A). Two trout held at 19 C to November showed complete retention of mature ova in the ovary. Large numbers of apparently mature ova (diameter > 3 mm) were in various stages of resorption. The greatest number of atretic ova were seen at 21 C ($N = 4$) in the November and December samples (Fig. 4B). Development of recruitment stock ova was noticeably reduced in the ovaries of fish held at 19 and 21 C.

Factors Influencing Embryo Survival

Spawning and incubation temperatures — The temperature of the water preceding spawning had a significant effect on gamete viability in the foregoing experiment. A 4 × 3 factorial experiment was conducted to determine the effects of both spawning and incubation temperatures on embyro survival to hatch. Analysis of variance indicates that both spawning and incubation temperatures and their interaction have a significant effect on percentage of normal hatch ($P < 0.005$, F-test). A plot of this interaction is illustrated in Fig. 5. No normal hatch occurred from spawnings at 16.1 C. Orthogonal comparisons reveal no difference in response between 10 C and Lake Superior spawning temperatures ($P > 0.10$). A 30% normal hatch could be expected if eggs were spawned at Lake Superior temperatures (10.7 C) and incubated at 14.6 C; however, a comparable normal hatch was obtained for spawnings at 13.2 C and incubated at 10.9 C. Rearing embryos at an optimum temperature is not sufficient for a good hatch, since the temperature history preceding spawning must also be optimal for maximum egg viability.

Incubation temperature — The lower and upper tolerance limits for normal hatch of several fish species corresponded to the range of temperatures observed on their spawning grounds (Hokanson et al. 1973). It was desired to estimate the upper T_L50 for normal hatch of brook trout for comparison with the upper E_T50 reported for viable egg production. The percentage total hatch and percentage normal hatch decreased with increasing incubation temperature (Table 4). No hatch occurred at 18 C. The incidence of abnormalities was highest at 15 C. A maximum mean normal hatch of 83% (range 77–90%) occurred at 6 C. Normalized percentage values for normal hatch and viable eggs per female are plotted against temperature in Fig. 2. The upper E_T50 for viable egg production (11.7 C) was significantly lower than the upper T_L50 of 12.7 C (1 SE = 0.14, N = 3) for normal hatch ($P < 0.05$, t-test), although the difference is only 1 C.

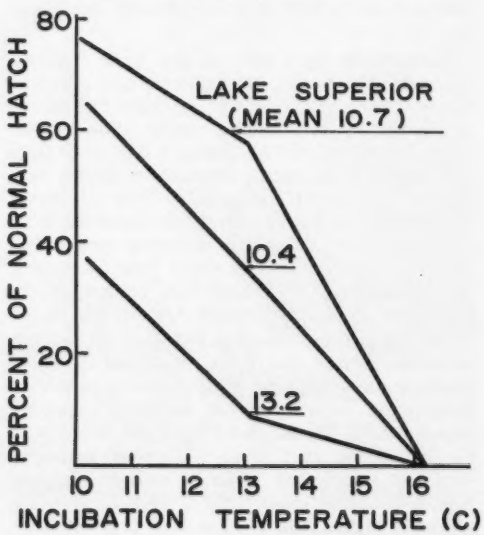

FIG. 5. Effect of incubation temperatures on percent normal hatch of brook trout at different spawning temperatures (Lake Superior, 10.7 C; 10.4 C; 13.2 C).

Incubation temperature, tempering method, age — A 4 × 3 × 2 factorial experiment was conducted to determine the effects of incubation temperature, tempering methods, and age of the embryo on normal hatch of brook trout. Incubation temperature was the most important factor influencing the percentage of normal hatch (Table 5). The age of the embryo is more important than the tempering method in determining the response of brook trout embryos to incubation temperature. Methods of tempering have no significant effect on this response. A greater percentage of embryos hatched normally when exposed to temperature extremes at the eyed stage than the earlier combined developmental stages. This response was significant only at 15 C where 22.3% of the eyed embryos hatched normally compared to 0.3% normal hatch for the other combined stages (Fig. 6). The upper TL50 for normal hatch increased from 13.3 C for the three younger stages to 14.1 C for the eyed stage of development.

TABLE 4. Brook trout embryo survival to hatch at five constant temperatures.

Nominal incubation temp. (*C*) (*Duplicate*)	No. of eggs	% total hatch	% normal[a] hatch
6 (*A*)	107	84	82
(*B*)	108	92	90
(*C*)	109	78	77
9 (*A*)	97	84	81
(*B*)	109	74	72
(*C*)	107	71	65
12 (*A*)	114	66	54
(*B*)	99	63	56
(*C*)	107	68	60
15 (*A*)	103	40	3
(*B*)	111	23	1
(*C*)	106	12	0
18 (*A*)	109	0	0
(*B*)	105	0	0
(*C*)	102	0	0

[a]Total hatch less dead and abnormal larvae at hatch.

Implications for Thermal Criteria

Studies on the relation of temperature to tolerance, preference, metabolic rate, performance, and growth of brook trout all point to a physiological optimum in the region of 13–19 C (Fry 1951). Brook trout stocked in ponds are known to survive when the deepwater temperature is below 21 C, but not above (Johnson 1967). Brook trout are known to survive higher temperatures for short periods (Fry et al. 1946). Natural occurrence of self-sustaining populations is limited at temperatures above 19–20 C (Creaser 1930; Fry 1951; Tarzwell 1957). Lower temperatures are required for natural occurrence of

TABLE 5. Analysis of variance for identification of important factors affecting brook trout embryo survival to hatch. Significant orthogonal comparisons are included. Embryos were transferred directly or tempered to 9, 12, and 15 C at water hardening (H), cleavage (C), gastrula (G), or eyed (E), developmental stages[a].

Source of variation	df	Sum of squares	Mean square	Calculated F-value	*P*[b]
Total	23	21,549.67	–	–	–
Developmental stage (D)	3	427.06	142.35	1.552	N.S.
E vs. (H+C+G)	1	401.96	401.96	4.482	(0.10)
Incubation temp. (T)	2	19,266.91	9,633.46	105.011	(0.005)
15° vs. (9°+12°)	1	18,828.22	18,828.22	210.022	(0.005)
9° vs. 12°	1	438.69	438.69	4.782	(0.10)
Method of transfer (M)	1	6.00	6.00	0.065	N.S.
D×T	6	815.38	135.90	1.481	N.S.
15°-E vs. 15°-(H+C+G)	1	709.07	709.07	7.729	(0.05)
D×M	3	318.45	106.15	1.157	N.S.
T×M	2	165.45	82.72	0.902	N.S.
D×T×M (error)	6	550.43	91.74	–	–

[a]Mean incubation temperature preceding transfer was 7.0 C.
[b]Probability level at which calculated F-value could have been derived by chance; N.S. denotes not significant at $P = 0.10$.

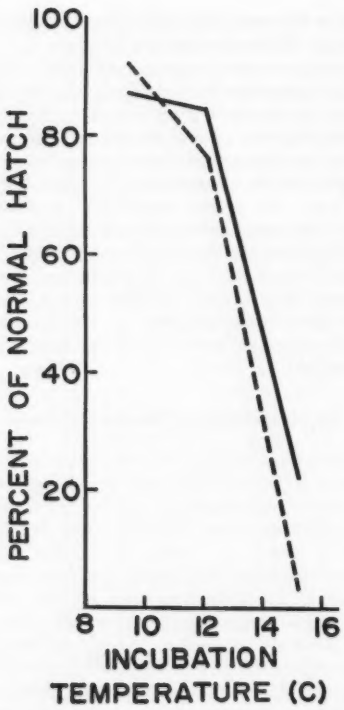

FIG. 6. Percentage of normal hatch of brook trout exposed to high incubation temperatures at the eyed stage (solid line) compared to the combined response of the water hardening, cleavage, and gastrula stages (dashed line). Initial incubation temperature of all stages was 7.0 C.

a breeding population when brook trout are competing with more heat tolerant species (King 1937; Vincent and Miller 1969).

In brook trout, secondary growth of ovaries and attainment of functional maturity of males will occur at 19 C, the apparent upper limit for the occurrence of natural breeding populations. The maturity indices for females at elevated temperatures were as large or larger than those reported by Vladykov (1956) for natural populations. Secondary growth of oocytes takes place at elevated temperatures (19–21 C), but ova could not be stripped from these fish. Henderson (1963) stripped female brook trout reared at 8 and 16 C to determine the onset of functional maturity. In this study, ova retention, high temperature, or both appeared to inhibit the normal development of recruitment stock ova at 19 C. The inhibition of spawning and subsequent resorption of mature ova for some species is known to interfere with the development of the recruitment stock ova for the next spawning season (Wallace 1903; Kukuradze 1968). Apparently the maximum temperature for normal maturation and ovulation for female brook trout lies between 16 and 19 C.

The maturity indices of male brook trout in November (mean 3.4 and 2.9%) at 19 and 21 C, respectively, were larger than those described for populations (1.5%) in an infertile stream by Wydoski and Cooper (1966). Growth of the testes was nearly completed at the start of the exposure and testes were in the rapid phase of spermatogenesis (stage 3) which contained germ cells at all stages of maturation. It could not be determined histologically if spermatogenesis was inhibited at 21 C, but no motile spermatozoa could be stripped from these fish during their normal breeding season. Maturity indices and cytological evidence showed that testes at 21 C were in a period of involution (stage 6) during the spawning season (November) and regression was proceeding faster than at 19 C. One week exposure of male trout to 21 C caused rapid disappearance of spermatozoa of functionally mature fish, but not those in earlier stages of spermatogenesis. Ahsan (1966) found that higher temperatures (16–21 C) caused rapid disappearance of spermatids and spermatozoa in prespawning lake chub, *Couesius plumbeus*, and induced a quick post spawning regression of the testes, followed by a slow proliferation of the spermatogonia. Lower temperatures (5–12 C) favored retention or survival of spermatozoa in the lobules and ducts, and in post spawning fish promoted rapid proliferation of primary spermatocytes from earlier stages of spermatogenesis. These observations suggest that 19 C is near the maximum temperature at which normal spermatogenesis takes place in brook trout.

The present study was conducted during the secondary growth phase of the ovaries; however, Henderson (1963) found that brook trout exposed to a constant 8 and 16 C from February till spawning developed normally and reached functional maturity by mid-October. The rate of gonadal development was the same at 16 C as it was at 8 C provided the fish were exposed to natural day lengths. Brook trout spawn in late August or in September in the northernmost parts of their range (60°N), and during November in the southernmost areas (35°N). Spawning occurred primarily in October and November in this study which was similar to Henderson's observations. We also found that temperature had little influence on the timing of the spawning season in this species. However, brook trout do attempt to spawn at elevated temperatures during their normal breeding season, but

with reduced success. It is not known if mature brook trout would avoid unfavorable spawning temperatures, but upstream migrations may be stimulated by cooler water. Apparently the seasonal temperature cycle must be in phase with the normal day-length cycle for successful reproduction in this species.

Attainment of functional maturity does not insure successful reproduction since the thermal requirements for spawning and embryo survival are distinctly lower. Spawning activity, production of viable eggs, and embryo survival are dependent on water temperature. The upper TL50 for normal hatch was 12.7 C and the upper ET50 for viable eggs spawned per female was 11.7 C. This close relationship between favorable spawning and incubation temperature also exists for other species (Hokanson et al. 1973).

Optimal temperatures for brook trout reproduction would be expected at lower temperatures since both spawning (parental exposure) and incubation temperatures determine the proportion of embryos that hatch. The age of the embryo when transferred to temperature extremes is an important factor affecting their thermal tolerance. The method of tempering embryos to incubation temperature has little effect. Similar results were observed for *Esox lucius* (Hokanson et al. 1973). The tolerance limit of eyed brook trout embryos was 1 C higher than earlier developmental stages. Older embryonic stages of spring spawning species have greater increases in thermal tolerance (Lillelund 1966; Kelley 1968).

These results suggest that the entire seasonal temperature cycle limits the natural occurrence of self-sustaining brook trout populations. Brook trout maturation takes place in the summer months at temperatures favorable for growth, but spawning takes place in the fall at lower temperatures and embryos develop during winter months. Maximum summer temperature should not exceed the upper incipient lethal temperature of 25 C for juvenile brook trout (Fry et al. 1946). Mean summer water temperature should not exceed 19 C. Brook trout can perhaps tolerate summer temperatures greater than 19 C for brief periods without permanent damage to gametogenesis, but the water temperature should not exceed a maximum of 19 C or a mean of 16 C for the month preceding spawning to insure that the fish become functionally mature. The duration of exposure to 16–19 C that will allow successful spawning below 10 C in the fall should be known for more accurate criteria. To stimulate successful spawning, the water temperature should not exceed 12 C during the normal breeding season in each locality. Mean water temperature below 9 C is required for optimal spawning activity, gamete viability, and embryo survival.

Temperatures should not exceed a mean of 16 C or a maximum of 20 C to protect alevins hatching in the spring (McCormick et al. 1972). Lower temperature criteria would likely be required in brook trout habitat where optimal production is desired, especially in streams with a limited food supply, more heat-tolerant species, limited spawning areas, and exploited or despoiled by man.

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AHSAN, S. N. 1966. Effects of temperature and light on the cyclical changes in the spermatogenetic activity of the lake chub, *Couesius plumbeus* (Agassiz). Can. J. Zool. 44: 161–171.

ANDERSON, R. O. 1964. A sugar-flotation method of picking trout eggs. Progr. Fish-Cult. 26: 124–126.

ATZ, J. W. 1957. The relation of the pituitary to reproduction in fishes, p. 178–270. *In* G. E. Pickford and J. W. Atz [ed.] The physiology of the pituitary gland of fishes. New York Zoological Society, New York, N.Y.

BRIDGES, C. H., AND J. W. MULLAN. 1958. A compendium of the life history and ecology of the eastern brook trout, *Salvelinus fontinalis* (Mitchill). Mass. Fish. Bull. 23: 1–38.

BRUNGS, W. A. 1971. Chronic effects of constant elevated temperature on the fathead minnow (*Pimephales promelas* Rafinesque). Trans. Amer. Fish. Soc. 100: 659–664.

COCHRAN, W. G., AND G. M. COX. 1964. Experimental designs. 2nd ed. John Wiley and Sons, Inc., New York, N.Y. 617 p.

CREASER, C. W. 1930. Relative importance of hydrogen-ion concentration, temperature, dissolved oxygen, and carbon-dioxide tension, on habitat selection by brook trout. Ecology 11: 246–262.

DAVIS, H. S. 1965. Culture and diseases of game fishes. University of California Press, Berkeley, Calif. 332 p.

DIXON, W. J. 1970. BMD Biomedical computer programs. Univ. Calif. Publ. Automatic Computation No. 2, University of California Press, Berkeley, Calif. 600 p.

EMBODY, G. C. 1934. Relation of temperature to the incubation period of eggs of four species of trout. Trans. Amer. Fish. Soc. 64: 281–289.

FRY, F. E. J. 1951. Some environmental relations of the speckled trout (*Salvelinus fontinalis*). Proc. N. E. Atl. Fish. Conf., May 1951. 29 p.

FRY, F. E. J., J. S. HART, AND K. F. WALKER. 1946. Lethal temperature relations for a sample of young speckled trout, *Salvelinus fontinalis*. Univ. Toronto Stud., Biol. Ser. No. 54, Publ. Ont. Fish. Res. Lab. No. 66: 1–35.

HENDERSON, N. E. 1962. The annual cycle in the testis of the eastern brook trout, *Salvelinus fontinalis* (Mitchill). Can. J. Zool. 40: 631–645.

1963. Influence of light and temperature on the reproductive cycle of the eastern brook trout, *Salvelinus fontinalis* (Mitchill). J. Fish. Res. Board Can. 20: 859–897.

HOKANSON, K. E. F., J. H. MCCORMICK, AND B. R. JONES. 1973. Temperature requirements for embryos and larvae of the northern pike, *Esox lucius* (Linnaeus). Trans. Amer. Fish. Soc. 102: 89–100.

JOHNSON, M. G. 1967. Summer temperature relations in stream-fed ponds in southern Ontario. Progr. Fish-Cult. 29: 21–26.

KELLEY, J. W. 1968. Effects of incubation temperature on survival of largemouth bass eggs. Progr. Fish-Cult. 30: 159–163.

KING, W. 1937. Notes on distribution of native speckled trout and rainbow trout in the streams of the Great Smoky Mountains National Park. J. Tenn. Acad. Sci. 12: 351–361.

KUKURADZE, A. M. 1968. Effect of ecological conditions in the spawning period on the sexual cycle of the pike-perch (*Lucioperca lucioperca* (L.)) in the Kiliya Delta of the Danube. Probl. Icthyol. Amer. Fish. Soc. 8: 463–466.

LECREN, E. D. 1951. The length–weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilus*). J. Anim. Ecol. 20: 201–219.

LILLELUND, V. K. 1966. Versuche zur Ebrütung der Eier vom Hecht, *Esox lucius* L., in Abhängigkeit von Temperatur und Licht. Arch. Fischereiwiss. 17: 95–113.

MCCORMICK, J. H., K. E. F. HOKANSON, AND B. R. JONES. 1972. Effects of temperature on growth and survival of young brook trout, *Salvelinus fontinalis*. J. Fish. Res. Board Can. 29: 1107–1112.

MCCORMICK, J. H., AND R. F. SYRETT. 1970. A controlled temperature apparatus for fish egg incubation and fry rearing. National Water Quality Laboratory, Duluth, Minn. 9 p.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York, N.Y. 481 p.

TARZWELL, C. M. 1957. Water quality criteria for aquatic life, p. 246–272. *In* Clarence M. Tarzwell [ed.] Biological problems in water pollution. U.S. Public Health Serv. Publ.

VINCENT, R. E., AND W. H. MILLER. 1969. Altitudinal distribution of brown trout and other fishes in a headwater tributary of the South Platte River, Colorado. Ecology 50: 464–465.

VLADYKOV, V. D. 1956. Fecundity of wild speckled trout (*Salvelinus fontinalis*) in Quebec lakes. J. Fish. Res. Board Can. 13: 799–841.

WALLACE, W. 1903. Observations on ovarian ova and follicles in certain teleostean and elasmobranch fishes. J. Microsc. Sci. 47: 161–213.

WYDOSKI, R. S., AND E. L. COOPER. 1966. Maturation and fecundity of brook trout from infertile streams. J. Fish. Res. Board Can. 23: 623–649.

Chemical Basis for Homing of Atlantic Salmon (*Salmo salar*) to a Hatchery

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Based on recoveries from traps situated at a hydroelectric dam and a hatchery 1500 m downstream, the return location of hatchery reared and wild Atlantic salmon is examined. During the fall runs of 1971 and 1972, 97% of ascending wild salmon returned to the dam; only 3% were recovered at the hatchery. Despite the fact that the hatchery discharge contributed only 1/1000th of the river's flow, 67% of the hatchery-reared fish returned to the hatchery and 33% to the dam.

Tank tests demonstrated a clear-cut preference by hatchery adults for diluted hatchery effluent vs. river water. Wild fish showed no preference to either water. Well water, a component of hatchery effluent, was avoided by both hatchery and wild fish. Addition of $CuSO_4$ to preferred water altered its effectiveness.

SUTTERLIN, A. M., AND R. GRAY. 1973. Chemical basis for homing of Atlantic salmon (*Salmo salar*) to a hatchery. J. Fish. Res. Board Can. 30: 985–989.

Nous analysons les retours de saumons atlantiques d'élevage et de saumons sauvages à partir de recaptures de trappes placées près d'un barrage hydroélectrique et près d'un établissement de pisciculture situé à 1500 m en aval. Au cours des montaisons d'automne de 1971 et 1972, 97% des saumons sauvages sont retournés au barrage; seulement 3% ont été recapturés à l'établissement de pisciculture. En dépit du fait que l'effluent de l'établissement de pisciculture ne contribue que 1/1000ième du débit de la rivière, 67% des poissons d'élevage sont retournés à la pisciculture et 33% au barrage.

Des essais en viviers démontrent une préférence bien marquée des adultes d'élevage pour l'effluent de l'établissement piscicole. Les poissons sauvages n'ont de préférence ni pour l'une ni pour l'autre eau. L'eau de puits, un composant de l'effluent de pisciculture, est évitée tant par les poissons d'élevage que par les poissons sauvages. L'addition de $CuSO_4$ à l'eau préférée en affecte l'efficacité.

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THE success of smolt-stocking programs in particular tributaries of a river system depends on whether or not adult salmon return to these tributaries to provide angling and progeny. Homing to natal rivers by wild salmon or rivers of release by hatchery-reared salmon, as opposed to straying to other rivers, by adult Atlantic salmon returning from the sea appears to be quite precise (Carlin 1968; Saunders 1969; Kerswill 1971; Ritter 1972). However, results of the few studies designed to examine homing to various tributaries within a river system by Atlantic salmon indicate that homing precision is variable (White 1936; Saunders 1969; Kerswill 1971). Atlantic salmon hatcheries in Sweden, Norway, Iceland, the United Kingdom, and one in the Canadian Maritime Provinces (Mactaquac Fish Culture Station) have taken advantage of this homing tendency as a means of assessing adult returns, procuring broodstock for artificial propagation, and distributing adult salmon to inaccessible spawning and nursery areas.

This study is an attempt to assess the precision of homing of hatchery-reared Atlantic salmon to a hatchery. The chemical factors responsible for the discriminatory behavior during adult migration are also examined.

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Materials and Methods

Smolt Releases and Adult Returns

Mactaquac Fish Culture Station and hydroelectric dam are located approximately 96 km inland, on the Saint John River, N.B. Hatchery-reared smolts which had an adipose fin clip were allowed to migrate of their own accord from the hatchery-rearing ponds down the migration channel and into the river (Fig. 1). Returning adult hatchery fish were recovered in a trap situated at the upstream end of the migration channel, or at the dam trap located 1500 m upstream from the hatchery. Unmarked wild smolts derived from rearing grounds above the headpond were similarly recovered as adults either at the hatchery trap or the dam trap. The distribution of wild and hatchery adults between these two traps was used as an index of homing precision.

Fig. 1. Site of Mactaquac dam and hatchery.

Tank Studies on Water Preference

Adult salmon used in the water-preference studies were transported by tank truck from the two field traps to the experimental pool. Some fish were held in well-water enclosures before being tested. Fish available during the fall run in 1971 were predominately grilse (1-sea-winter), but during the fall of 1972, nearly all fish tested were 2-sea-winter fish. As no difference in the homing behavior in the field or water preference in the experimental apparatus was noted as a result of age or sex, data for all salmon will be grouped together.

The experimental apparatus for testing water preference was a 4.5-m diameter plastic-lined swimming pool connected by pipes to two smaller tanks equipped with funnel traps made of netting (Fig. 2). This pool was situated in a field next to the hatchery aeration tower.

River or well water, obtained from the hatchery aeration tower, was directed into the tank traps at a flow rate of 60 liters/min resulting in a velocity of 4 cm/sec through the migration pipes. At velocities less than this the fish appeared to have difficulty locating the pipe entrance. Temperature of the river water decreased from 17 to 12 C during the period of testing. The attractiveness of well water compared to river water was examined only during those periods of the fall when temperatures of the two were within 1 C of each other. The O_2 content of river and well water was always greater than 75% of air saturation. River water was reddish brown due to humic acid content; well water was clear.

Hatchery effluent was pumped from the hatchery migration channel and transported in tanks to the testing apparatus. Effluent was siphoned into the flow of river water entering one side of the testing apparatus to give a final dilution of 1 part effluent to 1000 parts river water. This dilution was chosen as being realistic in terms of concentrations that might exist in the river downstream from the hatchery.

One pollutant, $CuSO_4$, was selected as a possible agent which might alter water preference. This was

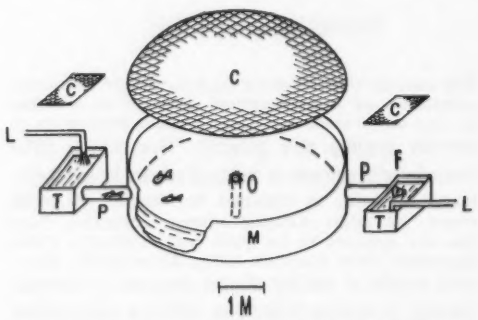

Fig. 2. Experimental pool: M, Main tank; F, Funnel traps; T, Trap tanks; L, Delivery line from aeration tower; O, Overflow; P, Migration pipe; C, Net covers.

similarly introduced to give a final concentration of 44 μg/liter. This level refers to that present in the tank trap and that passing through the migration pipes. Background levels of Cu^{++} already present in the river water were not measured.

During each experimental trial 8–12 fish were placed in the main tank and at the end of 12 hr the number of fish in each of the three compartments was noted. During preliminary trials it was found that nearly all movement from the main tank occurred during the night. Consequently, a nightly testing period (8:00 PM–8:00 AM) was adopted. During at least half of the tests the tank traps were examined every 2 hr with a dim flashlight; such periodic examinations revealed no instances of escape from the traps. During the daylight hours the fish remained on the bottom of the tank in the shadows along the edge of the pool; orientation to selected outflows was observed on several bright nights when it was possible to see the fish.

In order to control for possible asymmetry in the apparatus, the same fish were used in most tests on two successive nights, water conditions being rotated. Movement of fish was much reduced after two nights of testing and naive fish were substituted.

Results

Homing Precision in the Field

The distribution of recaptures between the two trapping sites indicates some homing of hatchery fish has occurred (Table 1). Two-thirds of the hatchery returns were recovered at the migration channel while less than 3% of the wild fish were recovered there. Homing of hatchery fish to the hatchery during the spring and summer runs in 1971 and 1972 was considerably less precise than the fall runs. However, since only fall-run salmon were used for experimentation, it seemed valid to consider their homing precision apart from that of spring and summer runs.

Water Selection in Tanks

Flow rates of 60 liters/min for each side of the apparatus were chosen in preference to higher or lower rates because about half of the fish moved into the traps when presented with river water on both sides (Table 2A).

There is a definite preference exhibited by hatchery fish for river water containing small quantities of hatchery effluent relative to river water alone (Table 2B). Wild fish, however, appear to be indifferent to hatchery effluent and exhibit no particular preference when offered a choice of river water alone vs. river water plus hatchery effluent (Table 2C).

Because hatchery effluent contains a variable proportion of well water, its effectiveness was tested against river water. Both hatchery-reared and wild fish showed a preference for river water; no fish was ever captured in the wellwater side (Table 2D and 2E). Sublethal concentrations of $CuSO_4$ (1.0 ILL for young salmon, Sprague 1964) when added with hatchery effluent resulted in some degree of avoidance by hatchery fish (Table 2E).

Effects of River Hydraulic Conditions on Homing

The relatively constant flow of hatchery effluent during the summer months of 1971 and 1972 was approximately 21 ft^3/sec. Water flow through the dam (based on 24-hr daily averages) fluctuated tenfold from 4000 to 40,000 ft^3/sec. These changes in discharge often occurred within a few days (N.B. Power Commission operators' reports). Flow from the hatchery would therefore contribute anywhere from 1/200th to 1/2000th of the river volume downstream from the hatchery. It was anticipated that such fluctuations in relative flow rates might account for the low homing precision by hatchery fish during the spring and summer runs. Analysis of homing at different hydraulic conditions showed that no single aspect of water flow could account for the seasonal variation in homing precision.

Discussion

Considering the inequality of discharge from the hatchery and dam (1:1000), it is not surprising that a third of hatchery fish did not return to the hatchery. Homing by Atlantic salmon has been observed in Swedish hatcheries located on river systems (H. Peterson personal communication) and also to an Icelandic hatchery which releases a relatively small discharge directly into the ocean (T. Gudjonsson personal communication). Although not associated with a hatchery, homing to a small brook that flows several meters from a pond directly into the sea has been observed in Norwegian salmon (D. Møller personal communication).

Various methods have been used to examine effects of chemical factors in water on the behavior of adult salmonids. The temporal progress of salmon through counting fences or fish ladders (Brett and Mackinnon 1954; Saunders and Sprague 1967) has been used successfully but many variables are difficult to control. Electrophysiologically recorded activity from the olfactory bulb may be useful as an assay technique (Hara 1970) but problems of specificity and discrimination often make interpretation difficult (Oshima et al. 1969; Sutterlin and Sutterlin 1971). Increased locomotor

activity, perhaps indicative of arousal or migratory activity, has been used successfully by Idler et al. (1961) for Pacific salmon. During the present investigation, however, such immediate arousal responses during addition of hatchery effluent were not observed in Atlantic salmon, perhaps demonstrating basic species differences.

The use of olfactory cues by salmonids in homing to and within rivers appears well established (Bertmar and Toft 1969; for reviews see Hasler 1971, Harden Jones 1968, and Hara 1970). An olfactory mechanism is most likely operative during this investigation to account for water selection both in the field and in the tank studies. One serious problem that may have influenced the results in the tank experiments is that of sensitization as a result of the previous chemical experience of the fish. Holding some wild and hatchery fish in well water before testing was an attempt to exclude this possibility. This procedure did not result in selection of well water by hatchery or wild fish, nor did it alter the preference of these fish compared to those

TABLE 1. Distribution of adult hatchery-reared and wild fish during the fall runs in 1971 and 1972.

	Hatchery-reared fish		Wild fish	
Year	Recovered at hatchery	Recovered at dam	Recovered at hatchery	Recovered at dam
1971	104 (71%)	42 (29%)	25 (2%)	1029 (98%)
1972	151 (65%)	82 (35%)	25 (2%)	1289 (98%)
Total	255 (67%)	124 (33%)	50 (2%)	2318 (98%)

TABLE 2. Selection of various waters by hatchery-reared and wild Atlantic salmon.

A. Hatchery-reared fish (river water both sides)			B. Hatchery-reared fish			C. Wild fish		
No. fish	Right	Left	No. fish	River water	River water plus hatchery effluent	No. fish	River water	River water plus hatchery effluent
6	3	2	7	0	3 (R_1)[a]	9	2	3 (R_1)[a]
6	1	2	7	0	4 (L_1)	9	3	1 (L_1)
10	4	3	10	1	4 (R_2)	11	3	1 (R_2)
10	3	4	10	1	4 (L_2)	11	4	2 (L_2)
14	6	4	11	1	6 (R_3)	10	2	1 (R_3)
46	17	15	11	0	5 (L_3)	10	3	3 (L_3)
			12	1	5 (R_4)	60	17	11
			12	1	4 (L_4)			
			80	5	35			

D. Hatchery-reared fish			E. Wild fish			F. Hatchery-reared fish		
No. fish	River water	Well water	No. fish	River water	Well water	No. fish	River water	River water plus hatchery effluent plus Cu^{+2}
6	4	0 (R_1)[a]	10	4	0 (R_1)[a]	10	4	0 (R_1)[a]
6	3	0 (L_1)	10	5	0 (L_1)	10	4	1 (L_1)
8	4	0 (R_2)	20	9	0	10	2	1 (R_2)
8	3	0 (L_2)				10	3	0 (L_2)
11	6	0 (R_3)				40	13	2
39	20	0						

[a]R and L indicate the side (right or left) of the apparatus in which the river water was modified or replaced. Subscripts denote the experimental group of fish used for two tests.

that were tested directly following transport from the collection facilities. Sensitization is therefore unlikely.

A thousandfold dilution of hatchery effluent could be detected in the tank studies, and based on discharges from the hatchery and dam, similar dilutions would occur in the river even if mixing were thorough. Although the tank studies were an attempt to simulate occurrences in the main river, there may be several differences in the type of orientation mechanisms employed. Because of the opposed direction of water flow and the relatively sharp gradients occurring in the tank compared to the river, the problem of sensory adaptation during orientation is probably more easily overcome in the tank studies compared to orientation in the field.

The exact nature or source of the active components responsible for the homing of hatchery fish is uncertain. Well water alone repelled both hatchery and wild fish and neither were acclimated to well water alone during their freshwater life. Hatchery water supplies are normally composed of 50% ground water and 50% river water as well as a multiplicity of components derived from decaying fish food, fish excretion, and other chemicals associated with hatchery operations; as single components or collectively these may be involved in olfactory recognition of the migration channel by hatchery fish. Results by White (1936) led him to postulate that the presence of young fish in a tributary might elicit some degree of straying of returning adults reared in other portions of the water shed. During the present study the fact that less than 3% of wild fish entered the hatchery, which must be a large source of odor from young fish, casts doubt on the importance of the general attractant effect of young fish.

Addition of $CuSO_4$ (1.0 ILL for young salmon, Sprague 1964) to hatchery effluent altered the attractiveness of it to hatchery fish. Disrupted migratory progress of salmon ascending a river was observed at levels of 0.4 ILL for Cu^{++} plus Zn^{++} (Saunders and Sprague 1967). Because such factors as water hardness, background levels of metals and humic acid content were not measured during the present study, the avoidance observed can serve only to demonstrate this procedure as a potentially useful assay technique.

Acknowledgments

We thank hatchery personnel at Mactaquac Fish Culture Station for their assistance in this study. In particular, the cooperation of G. B. Robbins, E. L. Robinson, W. Austin, A. Rideout, and G. Campbell is acknowledged. The assistance of N. Sutterlin in several aspects of the study is greatly appreciated.

BERTMAR, G., AND R. TOFT. 1969. Sensory mechanism of homing in salmonid fish. I. Introductory experiment on the olfactory sense in grilse of Baltic salmon (*Salmo salar*). Behaviour 35: 235–241.

BRETT, J. R., AND P. MACKINNON. 1954. Some aspects of olfactory perception in migrating adult Coho and Spring salmon. *J. Fish. Res. Board Can. 11: 310–318.*

CARLIN, B. 1968. Migrations of salmon. In Series of lectures, Atl. Salmon Assoc. Spec. Publ., Montreal, Canada.

HARA, T. J. 1970. An electrophysiological basis for olfactory discrimination in homing salmon: A review. J. Fish. Res. Board Can. 27: 565–586.

HARDEN JONES, F. R. 1968. Fish migration. St. Martin's Press, New York, N.Y. 325 p.

HASLER, A. D. 1971. Orientation and fish migration, p. 429–510. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology, Vol. VI, Academic Press, New York and London. 559 p.

IDLER, D. R., J. R. MCBRIDE, R. E. E. JONAS, AND N. TOMLINSON. 1961. Olfactory perception in migrating salmon. II. Studies on a laboratory bioassay for homestream water and mammalian repellent. Can. J. Biochem. Physiol. 39: 1575–1583.

KERSWILL, C. J. 1971. Relative rates of utilization by commercial and sport fisheries of Atlantic salmon (*Salmo salar*) from the Miramichi River, New Brunswick. J. Fish. Res. Board Can. 28: 351–363.

OSHIMA, K., E. W. HAHN, AND A. GORBMAN. 1969. Electroencephalographic olfactory responses in adult salmon to waters transversed in the homing migration. J. Fish. Res. Board Can. 26: 2123–2133.

RITTER, J. A. 1972. Salmon hatchery evaluation in the Maritime provinces — results of smolt tagging in 1968. Resour. Develop. Branch Fish. Serv., Dep. Environ., Halifax, N.S. Progr. Rep. 5: 22 p.

SAUNDERS, R. L. 1969. Contributions of salmon from the Northwest Miramichi River, New Brunswick, to various fisheries. J. Fish. Res. Board Can. 26: 269–278.

SAUNDERS, R. L., AND J. B. SPRAGUE. 1967. Effects of copper–zinc mining pollution on a spawning migration of Atlantic salmon. Water Res. 1: 419–432.

SPRAGUE, J. B. 1964. Lethal concentrations of copper and zinc for young Atlantic salmon. J. Fish. Res. Board Can. 21: 17–26.

SUTTERLIN, A. M., AND N. SUTTERLIN. 1971. Electrical responses of the olfactory epithelium of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 28: 565–572.

WHITE, H. C. 1936. The homing of salmon in Apple River, N.S. J. Biol. Board Can. 2: 391–400.

NOTES

Effects of Brief Temperature Increases on Larvae of the American Oyster (*Crassostrea virginica*)[1]

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DIAZ, R. J. 1973. Effects of brief temperature increases on larvae of the American oyster (*Crassostrea virginica*). J. Fish. Res. Board Can. 30: 991–993.

Growth of larvae of the American oyster (*Crassostrea virginica*) was not affected by brief temperature increases of 10 or 15 degrees C, but a 20 degree increase had a detrimental effect. Mortality increased significantly following all temperature increases. Setting rates of larvae receiving 15 or 20 degree increases were significantly lower. Growth rate, mortality, and setting also seemed to be affected by the age at which larvae received a thermal shock.

DIAZ, R. J. 1973. Effects of brief temperature increases on larvae of the American oyster (*Crassostrea virginica*). J. Fish. Res. Board Can. 30: 991–993.

Une brève élévation de température de 10 ou 15 degrés C n'affecte pas la croissance des larves de l'huître américaine (*Crassostea virginica*) mais une augmentation de 20 degrés a un effet nuisible. Les mortalités augmentent significativement après toutes les élévations de température. Le taux de fixation des larves est nettement plus bas lorsqu'elles sont soumises à une élévation de température de 15 ou 20 degrés. Le rythme de croissance et les taux de mortalité et de fixation semblent également affectés par l'âge auquel les larves subissent un choc thermique.

Received November 7, 1972

LITTLE has been published on tolerance of oyster larvae to brief temperature increases. Lutz et al. (1970) studied the effects of temperature change on the setting of American oyster (*Crassostrea virginica*) larvae and reported that an increase of 5 C (from 24 to 29 C) for 4 hr, administered to larvae of setting size, increased the percentage that set. This report describes effects of 10-, 15-, and 20-degree increases for 5 sec on larvae of *C. virginica* at an ambient temperature of 25 C. This temperature was chosen as ambient because it is the temperature near which the oyster spawns in Chesapeake Bay and is within the range (Davis and Calabrese 1964) of optimum growth.

Materials and methods — Larvae were obtained from adults spawned in the laboratory at the Virginia Institute of Marine Science, the adults having been obtained from the Rappahannock and Potomac rivers. Three broods of larvae were used.

Stock cultures of larvae were maintained at 25 C in 80-liter polyethylene containers at a concentration of five to 10 larvae per milliliter. Larvae were fed 20–25 ml of a pure culture of the flagellate *Monochrysis* sp. per liter every 24 hr. Water in the stock cultures was changed every 48 hr and an antibiotic (0.2 ml/liter of Twin Biotic, Vineland Poultar Co., Vineland, N.J.) was added at each water change to control bacteria.

All experimental and control groups were maintained at 25±1 C and a salinity of 20‰ except when being exposed to temperature increase. Larvae from 2 to 20 days old, in 2-day increments, were used. Two replicates were run for each combination of age and test temperature.

Temperature increases were effected with a heat exchanger consisting of an entrainment tube in a constant temperature bath. After the exchanger was calibrated for a particular test temperature, larvae were run through the entrainment tube at a concentration of five to 10 per milliliter. Once larvae were exposed for 5 sec to a nominal test temperature ±0.5 C, 3–4 liters of water containing exposed larvae were placed in a gallon jar and cooled to 25 C in 30 min by immersion of the jar in a cold-water bath. Control larvae were subjected to the same conditions as the experimental larvae except that no temperature increase was administered. As the number of surviving larvae in the experimental and control groups decreased, the concentration of larvae was maintained at five to 10 per milliliter by decreasing the amount of new water added at each water changing. All groups

[1]Contribution No. 536 from the Virginia Institute of Marine Science, Gloucester Point, Va. 23062, USA.

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TABLE 1. Mortalities (M) and mean length increments (MLI) for American oyster (*Crassostrea virginica*) larvae of various ages at 48 hr after three 5-sec increases in temperature above 25 C, and setting rates at 30 days. All values are means of two replicates; mortality and setting rates being based on 30,000–35,000 larvae in each replicate, and increments on 40.

Age of larvae when exposed (*days*)	Initial length (μ)	M (%)	MLI with 0 degree increase (%)	Set rate (%)	M (%)	MLI with 10 degree increase (%)	Set rate (%)	M (%)	MLI with 15 degree increase (%)	Set rate (%)	M (%)	MLI with 20 degree increase (%)	Set
2	70	2.9	11.0	3.4	16.3	9.5	1.1	28.2	11.0	0.6	43.6	4.4	0.0
4	78	2.3	20.2	6.8	32.0	18.6	6.6	64.1	16.6	4.7	48.7	6.7	1.4
6	100	4.3	9.6	4.3	22.6	9.4	6.6	41.0	4.0	1.6	52.4	3.2	0.6
8	110	3.5	11.0	4.0	31.5	15.8	5.5	51.8	14.8	1.8	71.1	1.8	0.0
10	118	6.6	11.2	16.7	26.3	17.9	23.6	41.1	12.6	9.5	60.5	3.5	2.3
12	125	5.2	17.2	8.6	16.1	14.9	8.6	49.2	10.5	3.4	70.4	7.7	0.1
14	135	6.1	21.0	11.8	28.1	18.0	10.6	68.1	16.9	1.1	81.6	11.8	0.1
16	150	8.5	16.4	7.2	38.9	19.4	6.2	52.7	14.0	2.0	69.8	6.8	0.3
18	175	8.4	16.4	4.9	37.9	20.1	5.0	57.3	15.2	3.2	47.9	3.8	0.2
20	190	7.4	16.6	4.2	48.8	14.2	5.6	52.1	6.0	0.5	87.4	0.1	0.2
Mean		5.5	15.1	7.2	29.8	15.8	8.0	47.8	12.2	2.8	63.3	5.1	0.5

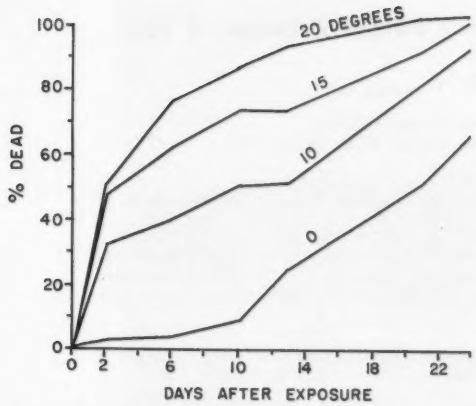

FIG. 1. Cumulative mortalities for 24 days of American oyster (*Crassostrea virginica*) larvae exposed for 5 sec to three increases in temperature above 25 C when 4 days old.

were maintained until all the larvae in a group died, set, or reached the age of 30 days.

To estimate growth and mortality, two 1-ml subsamples of larvae were taken from each gallon jar 48 hr after the temperature increase and then at 4-day intervals. The lengths, from anterior to posterior ends, of 40 live larvae were measured and all living larvae counted.

Analysis of covariance on the growth rates of the three broods of larvae used indicated that they were a homogeneous population (F for regression coefficient was 0.881).

Analysis of variance and Tukey's–*w* procedure (Sokal and Rohlf 1969) were used to determine if the test temperatures had significant effects on the larvae. No significant variation was found between any two replicate samples, the highest *t*-value between two replicates being 1.706, $n = 19$.

Mortality — Mortality was the most pronounced effect of the increases in temperature and was significantly higher ($\alpha = .01$) with each increase (Table 1). The mean 48-hr mortalities for exposed larvae ranged from 30 to 63% in comparison with 5.5% for larvae not exposed to an increase.

Greatest mortalities occurred in the first 4 days after a thermal shock, as illustrated by the data for larvae tested when 4 days old (Fig. 1). After this period, the mortalities of all groups were essentially the same.

Mortality increased, in general, with age of the larvae at time of exposure, e.g. the 48-hr mortalities of the 16- to 20-day-old larvae were 3, 31, 26, and 20% higher than for 2- to 6-day-old larvae for increases in temperature of 0, 10, 15, and 20 degrees. Three groups of larvae (2, 8, and 14 days old) given a 20 degree increase and 14-day-old larvae given a 15 degree increase all died before they were 30 days old. These deaths were attributed to some lasting effects of the higher temperature because none of the other groups exhibited premature death.

Growth — The growth of surviving larvae was influenced by the magnitude of the temperature increase. A 20 degree increase permanently impaired growth while increases of 10 and 15 degrees had no significant effect on surviving larvae. The average percent increase in length for larvae 48 hr after a temperature increase ranged from 16 to 5% as compared to 15% for the larvae not exposed to an increased temperature (Table 1). Temperature increases of 15 and 20 degrees slowed the growth of larvae for about 5 days, after which no consistent difference attributable to the test temperatures was found.

Setting — Setting was also influenced by the magnitude of the temperature increase and age of larvae (Table 1). Values for the 15- and 20-degree-increase groups at 30 days were significantly lower ($\alpha = 0.01$) than those for the 15- and 20-degree-increase groups, and those for the 20-degree group were lower than those for the 15-degree group. The mean setting range for exposed larvae was 8–0.5% compared with 7.2% for the larvae not exposed.

The age at which the larvae were exposed tended to affect setting, e.g. the values for 16- to 20-day-old larvae were lower than those for 2- to 6-day-old larvae after increases of 15 and 20 degrees, and higher after increases of 0 and 10 degrees. The increased set with age agrees with Lutz et al. (1970), who indicated that a decrease of less than 10 degrees from 24 C when the larvae are ready to set will increase setting.

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DAVIS, H. C., AND A. CALABRESE. 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. U.S. Fish Wildl. Serv. Fish. Bull. 63: 643–655.

LUTZ, R. A., H. HIDU, AND K. G. DROBECK. 1970. Acute temperature increase as a stimulus to setting in the American oyster *Crassostrea virginica* (Gmelin). Proc. Nat. Shellfish. Ass. 60: 68–71.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. W. H. Freeman and Co., San Francisco, Calif. 776 p.

Scolecobasidium humicola, a Fungal Pathogen of Fish

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Ross, A. J., and W. T. Yasutake. 1973. *Scolecobasidium humicola*, a fungal pathogen of fish. J. Fish. Res. Board Can. 30: 994–995.

Scolecobasidium humicola, a previously undescribed fungal pathogen of fish was isolated from coho salmon (*Oncorhynchus kisutch*). In natural infections the kidney was the organ most affected. The disease was difficult to transmit experimentally and appeared to be only weakly contagious.

Ross, A. J., and W. T. Yasutake. 1973. *Scolecobasidium humicola*, a fungal pathogen of fish. J. Fish. Res. Board Can. 30: 994–995.

Nous avons isolé du saumon coho (*Oncorhynchus kisutch*) un champignon pathogène inconnu jusqu'ici, *Scolecobasidium humicola*. Dans les cas d'infection naturelle, le rein est l'organe le plus affecté. La maladie se transmet difficilement dans des conditions expérimentales et ne semble que peu contagieuse.

Received May 24, 1972

This note records a previously undescribed pathological condition of coho salmon (*Oncorhynchus kisutch*) caused by *Scolecobasidium humicola*, a hyphomycete earlier described from soil (Barron and Busch 1962). The organism has been observed over the past few years in juvenile coho salmon held in our wet laboratory for experimental purposes. The disease is apparently not contagious, or only weakly so, as very few diseased fish have ever been seen in any group. Knowledge of the role of *S. humicola* as a parasite of cold-blooded animals was recently enhanced by the report of Elkan and Philpot (1973) who found this organism as a pathogen of frogs.

Materials and methods — Pathology of spontaneous infections — If external lesions (Fig. 1) were not present, the only external indication of disease was an enlarged abdomen. Internally, the body cavity usually contained a watery fluid. Adhesions frequently occurred between the various visceral tissues such as the liver, spleen, kidney, and adjacent muscle. Gray lesions in the organs appeared similar to those seen in fish with corynebacterial kidney disease.

The histopathologic cellular changes that occurred in the infected area were similar to those reported by Carmichael (1966) except that we found no mycetoma in the cerebral area. Numerous large lesions were present and these were heavily infiltrated with branching and fragmented hyphae and lymphoid cells (Fig. 2 and 3). Many of these areas also contained foci of degeneration and necrosis. The invasiveness of the infection was particularly evident in kidney tissue. No giant cells were seen, in contrast to the findings of Carmichael (1966).

Experimental infections — The disease was not transmitted by simply incorporating the fungus into the fish diet. However, success was achieved by adding ground glass to the feed as follows: The organism was grown on Sabouraud Dextrose Agar for about 7 days. The mat containing the mycelium with conidia was scraped from the surface of the agar, ground with water in a mortar and pestle, and mixed in a commercially prepared dry meal diet containing ground glass. The mixture was moistened with water, dried, and crumbled into pellets.

Ten 4- to 5-inch coho salmon held in dechlorinated municipal water at 18 C were fed the pellets twice, the second feeding 5 days after the initial feeding.

The first mortalities occurred about 1 month after exposure. At this time two fish were badly decomposed and one moribund specimen had several lesions visible on the body surface and extending into the muscle. This fish was killed but no fungus was seen in the internal organs, although all the surface lesions contained large numbers of hyphae. Deaths were sporadic and fungus was found in only one of the next four fish that died. Four months after exposure one of the three remaining fish became moribund and was examined. The only gross sign was swelling of the posterior kidney, where fungus was found. The two remaining fish were autopsied at this time, but no infection was seen.

Morphology of the fungus — Colonies of *S. humicola* on Sabouraud Dextrose Agar were strongly funiculose, heaped up in the center to form a dense mat. Young colonies were olivaceous in color with a powdery surface and a brown underside. Older colonies were olivaceous-

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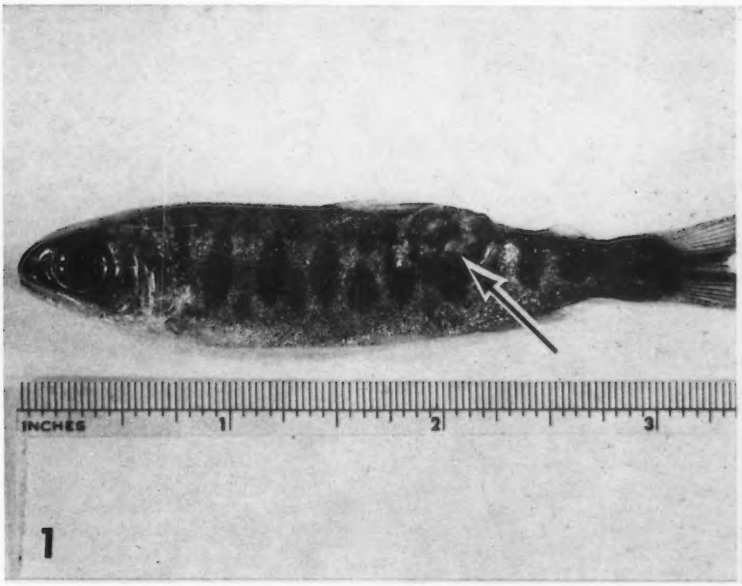

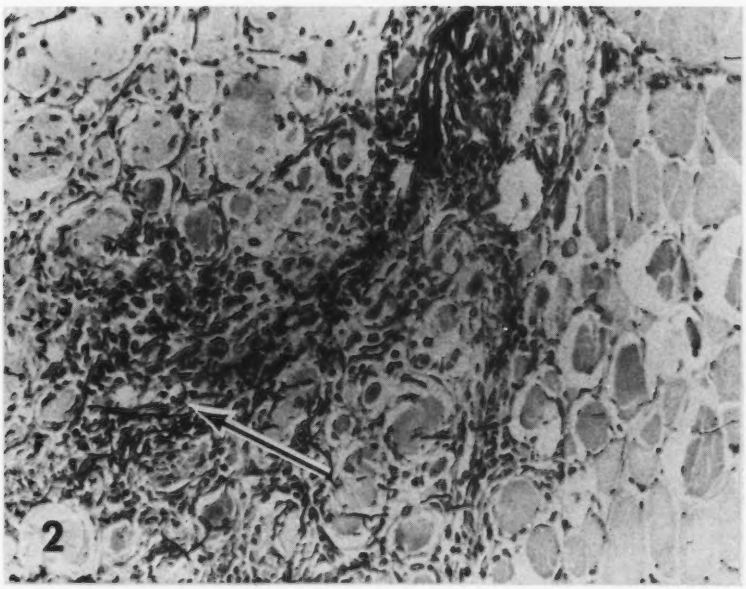

FIG. 1. Spontaneous infection of coho salmon caused by *Scolecobasidium humicola*.
FIG. 2. Histopathological changes occurring in the peripheral area of the external lesion shown in Fig. 1. Note hyphae and widespread inflammation in the infected area. May–Grunwald stain. × 700.

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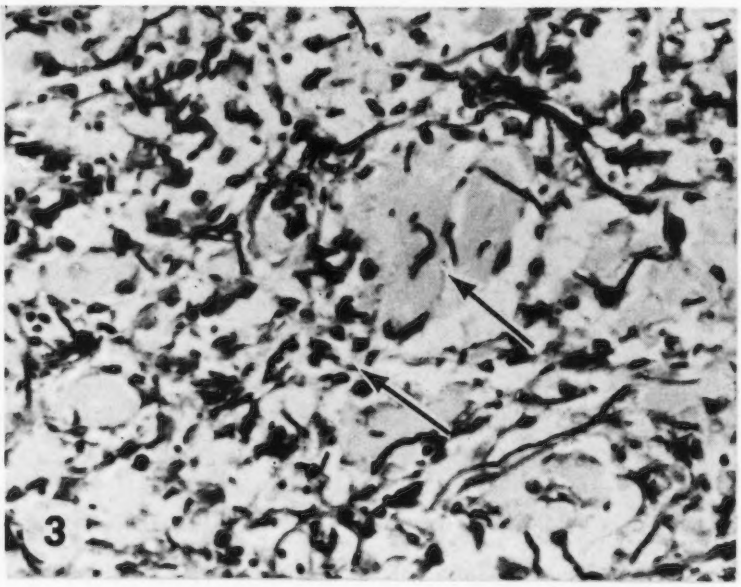

FIG. 3. Histopathological changes occurring in the peripheral area of the external lesion shown in Fig. 1. Note fragmented hyphae and widespread inflammation. May–Grunwald stain. × 1800.

FIG. 4. Slide culture of *S. humicola*. Note septate conidia. × 2700.

black with olive brown aerial hyphae. The colonies were dark brown to black in reverse and no visible exudate diffused into the medium. Colonial appearance on corn meal agar was similar, however, the colorations were less intense and the growth was not as dense.

A photomicrograph of a slide culture on Sabouraud Dextrose Agar is shown in Fig. 4. Conidiophores are borne on vegetative septate hyphae. The conidia characteristically have one septum, occur singly, and may be slightly echinulated.

Discussion — The most recent report of a systemic mycosis in fish is that of Carmichael (1966) who found cerebral mycetoma in cutthroat trout (*Salmo clarki*). Earlier, Doty and Slater (1946) studied a deep-seated mycosis of chinook salmon (*Oncorhynchus tshawytscha*) and identified the causative organism as *Heterosporium tshawytchae*. There are superficial similarities between *H. tshawytchae* and *Scolecobasidium*, and *S. constrictum* was originally described as *H. terrestre* (Barron and Busch 1962). These authors also point out that the genus *Heterosporium* has no taxonomic status. The possibility, therefore, exists that *H. tshawytschae* is also a species of *Scolecobasidium*.

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Barron, G. L., and L. V. Busch. 1962. Studies on the soil Hyphomycete *Scolecobasidium*. Can. J. Bot. 40: 77–84.

Carmichael, J. W. 1966. Cerebral mycetoma of trout due to a *Phialophora*-like fungus. Sabouraudia 5: 120–123.

Doty, M. S., and D. W. Slater. 1946. A new species of *Heterosporium* pathogenic on young chinook salmon. Amer. Midland Natur. 36: 663–665.

Elkan, E., and C. M. Philpot. 1973. Mycotic infections in frogs due to a *Phialophora*-like fungus. Sabouraudia. (In press)

Some Chemical Signals in the Spawning Behavior of Rainbow Trout (*Salmo gairdneri*)

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Newcombe, C., and G. Hartman. 1973. Some chemical signals in the spawning behavior of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 995–997.

In a two-choice maze ripe rainbow trout (*Salmo gairdneri*) of both sexes showed positive responses to chemical (probably olfactory) information. The possibility that visual or auditory stimuli influenced choice of channel in the maze was ruled out. Both males and females were strongly attracted to water taken from behind fish that were spawning and weakly attracted to the holding water of ripe fish that were not spawning. Brief speculation is offered about the role of chemical stimuli in the behavior of prespawning and spawning trout.

Newcombe, C., and G. Hartman. 1973. Some chemical signals in the spawning behavior of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 995–997.

Nous avons étudié les réactions de truites arc-en-ciel (*Salmo gairdneri*) dans un labyrinthe à deux choix. Les poissons réagissent positivement à un stimulus chimique (probablement olfactif). La possibilité que des stimuli visuels ou auditifs influencent le choix du chenal a été éliminée. Les poissons expérimentaux sont fortement attirés par l'odeur de poissons en voie de frayer, et faiblement attirés par l'odeur de poissons mûrs qui ne fraient pas activement. Nous examinons brièvement le rôle des stimuli chimiques ou olfactifs sur le comportement des truites avant et pendant la fraie.

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DURING studies on the reproductive biology of rainbow trout *Salmo gairdneri* (Hartman 1969 and 1970), it was observed that male trout appeared to detect readiness of the female to lay eggs without the necessity of prolonged visual contact. This raised the question of whether or not some nonvisual signal might be involved in helping to synchronize spawning behavior or bring groups of spawners together.

The role of chemical signals in reproductive behavior has been established for some other species of fish (e.g. *Poecilia reticulata*, Gandolfi 1969; *Colisa labia* and *C. labiosa*, Rossi 1969). Mate selection by the blind goby (*Typhlogobius californiensis*) is determined entirely by chemical signals (MacGinitie 1939).

This study was designed to determine whether a nonvisual, chemical stimulus might be involved in the spawning behavior of rainbow trout. The attempt was based on observation of responses of ripe males and females in a two-choice maze when various potential specific attractants were placed in one or other of two channels.

Materials and Methods — The two-choice maze (Fig. 1), constructed of plywood and painted dark brown inside, was covered with translucent panels between the start channel and the trap leads. Except in one test (Table 1), an opaque panel covered the maze upstream from the trap lead to induce fish to move upstream, but without bias for either channel of the maze. Trap leads were tapered to 4 cm in an effort to prevent fish from leaving a channel after entering it. Louvered baffles permitted waterflow through the maze channels but prevented test fish from seeing objects in the compartment where potential attractants were placed. No fish was used more than once.

Potential attractants were placed in one or other of the compartments for them on a randomized basis.

TABLE 1. Choices of ripe male and female rainbow trout (*Salmo gairdneri*) concerning various potential attractants.

Potential attractant	Test duration (*hr*)	Choices	
		Total	Positive (%)
	Test fish male		
1 F	0.5	10	63
	1.0	24	75
	2.0	34	71
	8.0	54	54
1 M	2.0	26	54
Egg wash	1.0	34	71
HW (50 F)[a]	8.0	16	56
SF[b]	8.0	27	82[d]
	Test fish female		
1 F	1.0	46	61[d]
	2.0[c]	13	23
	8.0	23	39
M/F	1.0	35	69
HW (40 F)	0.5	38	68
HW (80 M)	1.0	13	92[d]
SF	8.0	37	78[d]

[a]Holding water from group of 50 ripe females.
[b]Water taken downstream from spawning fish.
[c]Opaque panel not used.
[d]Probability that choices not due to chance < 0.05; chi-square test.

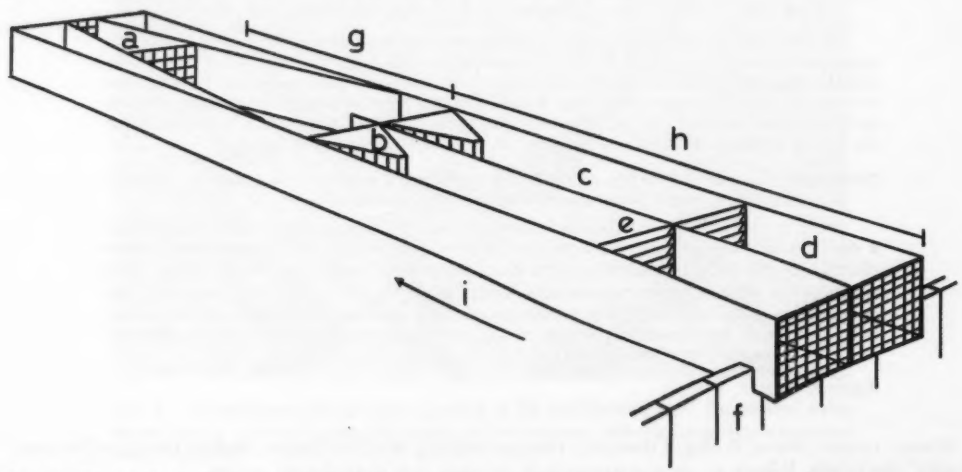

FIG. 1. Two-choice maze. a, start channel; b, trap lead; c, maze channel; d, compartment for potential attractant; e, louvered baffle; f, weir in stream; g, portion of maze covered by translucent panel; h, portion of maze covered by opaque panel; i, direction of water flow in maze. Maze dimensions: length, 6.2 m; width, 0.6 m; depth, 0.3 m.

If fish were used they were usually put in before the start of each test. The potential attractants tested were a single fish in one compartment or the other (1 F or 1 M, Table 1), a male in one compartment and a female in the other simultaneously (M/F, Table 1), or wash water from eggs (egg wash, Table 1) stripped from ripe females. Two hundred eggs (measured by volume) were agitated in 10 liters of water for 2 min. The wash water was decanted and diluted to 25 liters. This solution was prepared 5 min before the start of each test and drained into one of the appropriate compartments or the other at 25 liters/hr.

Holding water from groups of ripe fish (HW, 40 F, 50 F, or 80 M, Table 1). Water in which groups of 40-80 fish of the same sex were held was pumped through a polyethylene hose or other of the appropriate compartments during each test.
Water sampled directly downstream from actively spawning fish (SF, Table 1).

A minimum of four females and six males were placed in a penned section of stream. To have spawning occur continuously, spent females were replaced daily and males every second day. The water from behind the spawning fish was pumped into one or other of the appropriate compartments.

Test fish were placed in the start channel of the maze at least 5 min before the test. At the beginning of a test, the movable screen at the upstream end of the start channel was raised, releasing the test fish into the maze. Several arbitrarily selected test durations (0.5, 1, 2, and 8 hr) were used. At the end of the predetermined time period, the location of the test fish in the maze was recorded. Fish in the same channel as the potential attractant were said to have made a positive choice, and fish in the channel containing no potential attractant, a negative choice. Because few fish were in neither channel at the end of a test period, they were disregarded. In the test using a single male as a potential attractant in one compartment and a female in the other, fish were said to have made a positive choice when they were in the same channel as the female.

The experiments were conducted at Lloyds Creek (1969) and Loon Lake Hatchery (1970) in south central British Columbia.

In 1969 the fish were collected from streams near Kamloops. In May and June 1970 fish were obtained from a fish trap maintained by the British Columbia Fish and Wildlife Branch at Loon Lake Outlet Creek; in July and August fish were seined from Loon Lake Inlet Creek. The fish used ranged from 25 to 40 cm in length.

Results and Discussion — The choices of both males and females concerning the various potential attractants (Table 1) tended to be positive. In four tests the probability that the choices were due to chance was < 0.05 (chi-square test) for those of males to water taken downstream from spawning fish and for those of females to holding water from 80 adult males, to water taken downstream from spawning fish, and in the 1.0-hr test, to single females.

Two types of mechanical problems are considered to have influenced the results. In the test in which the opaque panel was not used (Table 1), tree shadows produced unequal light conditions in the channels of the maze. This may have accounted for the strong negative choice observed. In the 8-hr tests, some fish were known to have left the channel initially chosen, by way of the trap. If the potential attractant did not exert a strong influence on the behavior, the escapes may have influenced results.

Since both males and females were attracted to females, females to males, and both sexes to water associated with spawning fish, the actual attractants may not be specific to either sex. Furthermore, since the holding water alone and, also, the water from behind spawning fish attracted test fish, the stimulus involved could not be visual or auditory. It is likely that a chemical stimulus was involved and we speculate that it was olfactory.

This study does not indicate clearly whether rainbow trout can distinguish sexes by such chemical information. The question of chemical stimuli attracting males to females as signals of readiness to lay eggs was not answered. However, the role of such chemical information may be important in bringing scattered males and females together in a stream during the spawning season. Such information may have a further function in keeping groups of spawners together during nocturnal spawning.

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GANDOLFI, G. 1969. A chemical sex attractant in the guppy, *Poecilia reticulata* (Pisces, Poeciliidae). Monit Zool. Ital. (n.s.) 3: 89–98.

HARTMAN, G. F. 1969. Reproductive biology of the Gerrard stock of rainbow trout, p. 53–67. *In* T. G. Northcote [ed.] Symposium of salmon and trout in streams. H. R. MacMillan Lectures in Fisheries. Inst. Fish., Univ. of British Columbia, Vancouver, B.C. 388 p.

MACGINITIE, G. E. 1939. The natural history of the blind goby *Typhlogobius californiensis*. Amer. Midland Natur. 21: 489–505.

ROSSI, A. 1969. Chemical signals and nest-building in two species of *Colisa* (Pisces, Anabantidae). Monit. Zool. Ital. (n.s.) 3: 225–237.

Relation Between Simple Dynamic Pool and Surplus Production Models for Yield from a Fishery[1]

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JENSEN, A. L. 1973. Relation between simple dynamic pool and surplus production models for yield from a fishery. J. Fish. Res. Board Can. 30: 998–1002.

Dynamic pool models without self-regenerating properties are continuous age models, and surplus production models are continuous time models. Self-regenerating dynamic pool models are continuous age-discrete generation models and, also, discrete time-discrete age models. In a steady state specification of the regulatory function and direct estimation of biomass results in the surplus production model. Estimation of biomass by specifying the functions with respect to age for size of a cohort and individual weight and application of the coefficient of fishing mortality result in the dynamic pool model. A third approach, not applied in fisheries, is to specify the regulatory function and functions with respect to age of cohort size and individual growth in weight. In a steady state all methods for calculating yield give the same results if the functions specified are realistic. Specification of the functions requires that many assumptions be made. The dynamic pool model may be more accurate than the surplus production model because the regulatory function may be more difficult to determine than the functions with respect to age of cohort size and growth in individual weight.

JENSEN, A. L. 1973. Relation between simple dynamic pool and surplus production models for yield from a fishery. J. Fish. Res. Board Can. 30: 998–1002.

Des modèles pools dynamiques dépourvus d'autorégénération sont des modèles à âge continu, alors que les modèles surplus-production sont des modèles à temps continu. Les modèles pools dynamiques à autorégénération sont des modèles à âge continu-génération discontinue; ils sont également des modèles à temps discontinu-âge discontinu. Dans un état d'équilibre, la spécification de la fonction régulatrice et l'estimation directe de la biomasse aboutissent au modèle surplus-production. Par ailleurs, l'estimation de la biomasse par spécification des fonctions qui ont trait à l'âge correspondant à la taille d'une cohorte et au poids individuel d'une part, et l'application du coefficient de mortalité due à la pêche d'autre part, aboutissent au modèle pool dynamique. Une troisième méthode, non appliquée aux pêches, consiste à spécifier la fonction régulatrice et les fonctions qui ont trait à l'âge correspondant à la taille d'une cohorte et à la croissance pondérale individuelle. Dans un état d'équilibre, toutes les méthodes de calcul de rendement donnent les mêmes résultats, pourvu que les fonctions spécifiées soient réalistes. La spécification des fonctions requiert l'élaboration de plusieurs hypothèses. Le modèle pool dynamique peut être plus précis que le modèle surplus-production, parce que la fonction régulatrice est parfois plus difficile à établir que les fonctions qui ont trait à l'âge correspondant à la taille de la cohorte et à la croissance pondérale individuelle.

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MATHEMATICAL models applied to fish populations have been grouped into two basic categories: dynamic pool models such as those of Baranov (1918), Thompson and Bell (1934), Ricker (1944), and Beverton and Holt (1957); and surplus production models such as those of Hjort et al. (1933), Graham (1935), and Schaefer (1954, 1957). These two types of models have been compared by Schaefer and Beverton (1963) and by Silliman (1971).

These models differ in a fundamental way that does not appear to have been explicitly discussed in the literature. In dynamic pool models without self-regenerating properties the variables are continuous functions of age, whereas in surplus production models the variables are continuous functions of time. In this note the dynamic pool model without self-regeneration properties and the surplus production model will be reviewed with attention drawn to their time and age properties, and then the models will be compared.

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Mathematical symbols used are:

$B(t)$ = population size in biomass at time t;
B_∞ = environmental carrying capacity in terms of biomass;
B = annual biomass accumulation;
$f(B(t))$ = regulatory function that gives instantaneous rate of change in biomass;
F = fishing mortality coefficient;
F_1 = coefficient of effect of fishing on population density;
F_2 = coefficient of effect of fishing on average individual weight;
g = coefficient of growth in weight per individual averaged over the entire population;
K = coefficient of individual fish growth in weight or length;
k = coefficient of population growth in number of individuals and weight;
$N(x,t)$ = number of individuals of age x at time t;
$N(x)$ = number of individuals of a cohort alive at age x;
N_∞ = environmental carrying capacity in terms of the number of individuals;
$N(t)$ = total number of individuals in population at time t;
$P(t)$ = population size at time t in biomass or numbers;
R = recruitment;
r = coefficient of growth in number of individuals;
t = time measured in years;
v = greatest age attainable by an individual fish;
$W(x,t)$ = average weight of an individual of age x at time t;
$W(x)$ = average weight of an individual of age x;
W_∞ = maximum attainable weight of an individual fish;
$\overline{W}(t)$ = average weight of all individuals in the population at time t;
x = age measured in years;
x_0 = theoretical age at which length equals zero;
Y = yield in weight;
Y_E = equilibrium yield in weight;
Z = total instantaneous mortality coefficient.

In the above list, symbols of the form $B(t)$ indicate that B is a function of t and should not be confused with multiplication.

Review of typical dynamic pool model — The dynamic pool model for change in yield with respect to age is given by

$$\frac{dY}{dx} = F\,N(x)\,W(x)\,dx \qquad (1)$$

where F is a constant and $N(x)$ and $W(x)$ are unspecified functions of age. Change in the number of individuals in a cohort with respect to age is given by

$$\frac{dN}{dx} = -Z\,N(x) \qquad (2)$$

which after integration becomes

$$N(x) = R\,e^{-Zx}. \qquad (3)$$

A number of different models have been applied to describe weight as a function of age; Richards (1959) has shown that these models are related. Von Bertalanffy's equation

$$W(x) = W_\infty\,(1 - e^{-K(x-x_0)})^3 \qquad (4)$$

has frequently been applied for this purpose and appears to adequately describe the growth of many species of fish. Substitution of equations (3) and (4) into equation (1) gives the simple Beverton and Holt yield equation

$$\frac{dY}{dx} = F\,R\,e^{-Zx}\,W_\infty\,(1 - e^{-K(x-x_0)})^3. \qquad (5)$$

This model has recently been discussed in considerable detail by Gulland (1969). In equations (3), (4), and (5) population density, weight, and yield are all clearly functions of age. Under steady state conditions it has been shown that yield per generation equals annual yield from an entire population (Thompson and Bell 1934; Beverton and Holt 1957). In this situation annual yield can be calculated using equation (5), but it is constant with respect to time. In practice, to compensate for variation in recruitment, equation (5) is usually divided by recruitment to obtain an equation for yield per recruit (Gulland 1972). In most fisheries variation in recruitment is important and has led to investigation of stock–recruitment relations (Ricker 1958).

Self-regeneration properties of dynamic pool models, such as those proposed by Ricker (1954) and Beverton and Holt (1957), produce a continuous age-discrete generation model. These self-regenerating dynamic pool models usually have been applied to obtain yield per generation (Ricker 1958; Larkin and Ricker 1964; Tautz et al. 1969; Waller et al. 1971). A simple bookkeeping procedure can easily be established to obtain yield per year from a population consisting of several age-groups (Walters 1969). Continuous age models such as the Von Bertalanffy growth equation may be applied to calculate the constants in self-regenerating dynamic pool models, but these continuous age-discrete generation models are also discrete time-discrete age models as is clearly revealed by the nature of Walters' (1969) computer program. Self-regenerating dynamic pool models may offer the best approach for obtaining realistic models. However, these models are complex and are difficult to compare with the simpler models; their inclusion in this study would result in an undesirable degree of complexity.

Review of typical surplus production model — The general form of this model for an exploited population under average environmental conditions was formulated by Schaefer (1954) as

$$\frac{dP}{dt} = P(t)\,f[P(t)] - F\,P(t). \qquad (6)$$

If $P(t)$ is taken as biomass, then

$$P(t) = B(t) = N(t)\,\overline{W}(t) \tag{7}$$

where $N(t)$ is the total number of individuals of all ages at any point in time

$$N(t) = \int_0^v N(x,t)\ dx \tag{8}$$

and $\overline{W}(t)$ is the average weight of an individual at time t

$$W(t) = \frac{\int_0^v N(x,t)\ W(x,t)\ dx}{\int_0^v N(x,t)\ dx}. \tag{9}$$

It can be shown (Jensen 1972), by applying the equation

$$\frac{dB}{dt} = \overline{W}(t)\ \frac{dN}{dt} + N(t)\ \frac{d\overline{W}}{dt} \tag{10}$$

which is obtained by differentiating equation (7), that, if change in biomass with respect to time is given by the linear surplus production model

$$\frac{dB}{dt} = k\ B(t)\left(\frac{B_\infty - B(t)}{B_\infty}\right) - F\ B(t) \tag{11}$$

then suitable equations for $\frac{dN}{dt}$ and $d\overline{W}/dt$ are given by

$$\frac{dN}{dt} = rN(t) - \frac{rN(t)\ ^2\overline{W}(t)}{B_\infty} - F_1N(t) \tag{12}$$

$$\frac{d\overline{W}}{dt} = g\overline{W}(t) - \frac{gW(t)\ ^2N(t)}{B_\infty} - F_2\overline{W}(t) \tag{13}$$

where $k = r + g$. Yield with respect to time is given by the equation

$$\frac{dY}{dt} = F\ N(t)\ \overline{W}(t). \tag{14}$$

Substitution of equations (12) and (13) into equation (14) gives

$$\frac{dY}{dt} = F \int_{\Delta t}\left[rN(t) - \frac{rN(t)^2\ \overline{W}(t)}{B_\infty} - F_1N(t)\right] dt \cdot \int_{\Delta t}\left[g\overline{W}(t) - \frac{g\overline{W}(t)^2\ N(t)}{B_\infty} - F_2\overline{W}(t)\right] dt. \tag{15}$$

Clearly, equations (12), (13), and (15) give population size, average individual weight, and yield as functions of time.

Comparison of simple dynamic pool and surplus production models under steady state conditions — Virtually no exploited fish population is in a steady state but, as a first approximation in modeling a fish population, a steady state is usually assumed (Gulland 1972). In the steady state the difference between dynamic pool and surplus production models results from specifying different functions. In a steady state, where $dB/dt = 0$, $dN/dt = 0$, and $d\overline{W}/dt = 0$, annual yield from a fishery is given by

$$Y_E = F\ B = B\ f(B) \tag{16}$$

which is obtained from equations (6) and (7). Biomass accumulation of a cohort during its lifetime in a steady state is given by

$$\int_0^v N(x)\ W(x)\ dx \tag{17}$$

where $N(x)$ and $W(x)$ are unspecified functions of age. Biomass accumulation from an entire population during 1 year of life under steady state conditions is given by

$$\int_0^1 N(t)\ W(t)\ dt \tag{18}$$

but $N(t)$ and $\overline{W}(t)$ are constant with respect to time. Substituting equations (8) and (9) into expression (18) gives

$$\int^1 \int_0^v N(x)\ dx \left(\frac{\int_0^v N(x)\ W(x)\ dx}{\int_0^v N(x)\ dx}\right) dt \tag{19}$$

and, after cancellation of similar terms and integration with respect to time from 0 to 1, expression (19) becomes the same as expression (17). Under steady state conditions the biomass accumulation of a cohort during its lifetime equals the biomass accumulation of an entire population during 1 year.

Substitution of expression (17) into equation (16) for annual biomass accumulation gives

$$Y_E = F \int_0^v N(x)\ W(x)\ dx = f(B) \cdot \int_0^v N(x)\ W(x)\ dx. \tag{20}$$

From equations (16) and (20) it is clear that yield in a steady state can be calculated by four different approaches:

(1) Specify the form of no functions and apply the equation

$$Y_E = F\ B. \tag{21}$$

This method was applied by Ricker (1945, 1958), who separated the life of the fish into segments and applied equation (21) to each segment and then summed over the segments to obtain yield from the entire population.

(2) Specify the form of the regulatory function, $f(B)$, and apply the equation

$$Y_E = f(B)\, B. \tag{22}$$

This is the surplus production approach. Examples of the application of these models to specific fisheries are given by Ricker (1958), Schaefer (1954), and Gulland (1972). Ricker reviews the assumptions of this approach.

(3) Specify the form of $W(x)$ and $N(x)$ and apply the equation

$$Y_E = F \int_0^v N(x)\, W(x)\, dx. \tag{23}$$

This is the dynamic pool approach. Examples of the application of these models are given by Gulland (1972), Beverton and Holt (1957), and Gulland (1969). Ricker (1958) and Beverton and Holt (1957) review the assumptions of this approach.

(4) Specify the form of $f(B)$, $N(x)$, and $W(x)$ and apply the equation

$$Y_E = f(B) \int_0^v W(x)\, N(x)\, dx. \tag{24}$$

Equation (24) has not been applied. Application of equation (24) is equivalent to applying both the dynamic pool and surplus production models to the same fish population. Gulland (1972) has applied both the linear surplus production model and the Beverton and Holt yield equation to the eastern Pacific yellowfin tuna fishery, but he did not combine the two models into a single equation. Equation (24) combines the surplus production concept of the environmental carrying capacity with the age-specific events of the dynamic pool model. Application of equation (24) requires many of the assumptions of both equations (22) and (23). Equation (24) may not be of practical value, but it is of interest for it shows that in a steady state the constant for fishing mortality, F, of the dynamic pool model equals the regulatory function, $f(B)$, of the surplus production model.

All four expressions for yield are equal and theoretically give the same result. However, in fitting specific models to data different results may be obtained with the four yield equations if the functions selected for $f(B)$, $N(x)$, and $W(x)$ are not realistic. For example, if biomass is calculated by functions $N(x)$ and $W(x)$ that poorly approximate mortality and growth, the annual biomass accumulation estimated directly from the weight of the catch may not equal the annual biomass accumulation calculated by expression (17). Therefore, yield calculated by the surplus production approach (equation 22) may not equal yield calculated by the dynamic pool approach (equation 23).

The dynamic pool yield equation may be more accurate than the surplus production yield equation because the form of the functions $N(x)$ and $W(x)$ can be determined more easily than the form of the function $f(B)$. To obtain data on the form of $W(x)$ and $N(x)$ in a steady state, either observations can be made throughout the lifetime of a cohort or observations can be made on the entire population at a single point in time. To obtain data on the form of $f(B)$, estimates of yield and biomass are required for different steady states. The dynamic pool model must be used if the parameters it contains, such as age of entry into the fishery, are of interest. Gulland (1972) has discussed in considerable detail the practical aspects of applying both the dynamic pool and surplus production models.

Schaefer and Beverton (1963, equation 1) give a general equation that describes the dynamics with respect to time of an exploited fish stock. The terms in this equation for changes with respect to time were confused with the terms for changes with respect to age that are contained in the expressions for biomass in both the dynamic pool and the surplus production models. This led to the incorrect conclusion that the dynamic pool model describes a population in terms of recruitment, growth, and natural and fishing mortality, whereas the surplus production model combines these effects into a common function of the mean population size. Equation (23) shows that yield calculated by the dynamic pool model is the product of the fishing mortality coefficient and annual biomass accumulation. Annual biomass accumulation is estimated analytically using functions $N(x)$ and $W(x)$. In the dynamic pool model the terms for recruitment, growth, and natural and fishing mortality are contained in the expression for annual biomass accumulation. Equation (22) shows that yield calculated by the surplus production model is the product of the regulatory function and annual biomass accumulation. In practice annual biomass accumulation is estimated directly from the biomass of the catch without aid of the functions $W(x)$ and $N(x)$. In the surplus production model the terms for recruitment, growth, and natural and fishing mortality are all combined in the expression for annual biomass accumulation. The regulatory function is a function of biomass; therefore, the regulatory function is also a function of recruitment,

growth, and natural and fishing mortality. In addition, however, the regulatory function contains terms that have no counterpart in the dynamic pool model. These terms are the coefficient for growth in number of individuals with respect to time, r; the coefficient of growth of average individual weight, g; and the environmental carrying capacity in terms of biomass, B_∞. The regulatory function is a complex function.

In summary, the surplus production model is a function of time and the simple dynamic pool model is a function of age. In the steady state the differences between the dynamic pool and surplus production models result from specifying different functions: (1) In the dynamic pool model analytical functions are applied to determine biomass. In surplus production models biomass is estimated directly from the weight of the catch; (2) In dynamic pool models the proportion of the annual biomass accumulation that is captured by fishing is estimated directly by the coefficient of fishing mortality. In surplus production models the proportion of the annual biomass accumulation that is captured by fishing is equated to the regulatory function. The regulatory function gives the rate of growth of biomass per unit of biomass that would occur in the absence of fishing. If fishing were stopped, biomass would increase to the environmental carrying capacity, and the rate of biomass increase at the moment fishing stopped would equal the coefficient of fishing mortality. The dynamic pool and surplus production models are not based on mutually exclusive theories, but when specific functions are defined the results calculated by these two methods may not agree. The dynamic pool and surplus production approaches are only two of four possible approaches for calculating yield under steady state conditions. Some of the four approaches may be more accurate than others.

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BARANOV, F. I. 1918. On the question of the biological basis of fisheries [in Russian]. Nauch.-Issled. Ikhtiol. Inst. Izv. 1: 81–128.

BEVERTON, R. J. H., AND S. J. HOLT. 1957. On the dynamics of exploited fish populations. H.M.S.O., London. 533 p.

GRAHAM, M. 1935. Modern theory of exploiting a fishery, and application to North Sea trawling. J. Cons. Cons. Perma. Int. Explor. Mer 10: 264–274.

GULLAND, J. A. 1969. Manual of methods for fish stock assessment. Part 1. Fish population analysis. FAO Man. Fish. Sci. No. 4, Rome. 154 p.

1972. Population dynamics of world fisheries. University of Washington Press, Seattle, Wash. 336 p.

HJORT, J., G. JAHN, AND P. OTTESTAD. 1933. The optimum catch. Hvalradets Skr. 7: 92–127.

JENSEN, A. L. 1972. Population biomass, number of individuals, average individual weight, and the linear surplus-production model. J. Fish. Res. Board Can. 29: 1651–1655.

LARKIN, P. A., AND W. E. RICKER. 1964. Further information on sustained yields from fluctuating environments. J. Fish. Res. Board Can. 20: 647–678.

RICHARDS, F. J. 1959. A flexible growth function for empirical use. J. Exp. Bot. 10: 290–300.

RICKER, W. E. 1944. Further notes on fishing mortality and effort. Copeia 1944: 23–44.

1945. A method of estimating minimum size limits for obtaining maximum yield. Copeia 1945: 84–94.

1954. Stock and recruitment. J. Fish. Res. Board Can. 11: 559–623.

1958. Maximum sustained yields from fluctuating environments and mixed stocks. J. Fish. Res. Board Can. 15: 991–1006.

SCHAEFER, M. B. 1954. Some aspects of the dynamics of populations important to the management of commercial marine fisheries. Bull. Inter-Amer. Trop. Tuna Comm. 1: 27–56.

1957. A study of the dynamics of the fishery for yellowfin tuna in the eastern tropical Pacific Ocean. Bull. Inter-Amer. Trop. Tuna Comm. 2: 247–268.

SCHAEFER, M. B., AND R. J. H. BEVERTON. 1963. Fishery dynamics — their analysis and interpretation, p. 464–483. *In* M. N. Hill [ed.] The sea. John Wiley & Sons, New York, N.Y.

SILLIMAN, R. P. 1971. Advantages and limitations of "simple" fishery models in light of laboratory experiments. J. Fish. Res. Board Can. 28: 1211–1214.

TAUTZ, A., P. A. LARKIN, AND W. E. RICKER. 1969. Some effects of simulated long-term environmental fluctuations on maximum sustained yield. J. Fish. Res. Board Can. 26: 2715–2726.

THOMPSON, W. F., AND F. H. BELL. 1934. Biological statistics of the Pacific halibut fishery. (2) Effects of changes in intensity upon total yield and yield per unit of gear. Rep. Int. Fish. Comm., No. 8. Seattle, Wash. 49 p.

WALLER, W. T., M. L. DAHLBERG, R. E. SPARKS, AND J. CAIRNS, JR. 1971. A computer simulation of the effects of superimposed mortality due to pollutants on populations of fathead minnows (*Pimephales promelas*). J. Fish. Res. Board Can. 28: 1107–1112.

WALTERS, C. J. 1969. A generalized computer simulation model for fish population studies. Trans. Amer. Fish. Soc. 98: 505–512.

Trophic Analysis of Juvenile Pink and Chum Salmon (*Oncorhynchus gorbuscha* and *O. keta*) in Puget Sound

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Pink and chum salmon (*Oncorhynchus gorbuscha* and *O. keta*) fry and Clarke–Bumpus plankton tows were collected from three beach areas in Puget Sound in spring 1970. Chum fry and benthic pump samples were taken in 1971. The diets of the young of the two species were similar. Epibenthic harpacticoid copepods were the chief prey of the chum and pink salmon (57 and 36%, respectively, in 1970). Distinct differences were apparent, the more notable being the preference for invertebrate eggs exhibited by the pinks and the higher preferences for small gammarid amphipods and harpacticoids exhibited by the chums. The stomach contents showed no resemblance to the plankton hauls taken in the same area. The onshore stage of development appears to be a distinct ecological stage in the life cycles of these species.

KACZYNSKI, V. W., R. J. FELLER, J. CLAYTON, AND R. J. GERKE. 1973. Trophic analysis of juvenile pink and chum salmon (*Oncorhynchus gorbuscha* and *O. keta*) in Puget Sound. J. Fish. Res. Board Can. 30: 1003–1008.

Au printemps 1970, nous avons capturé des alevins de saumons rose et keta (*Oncorhynchus gorbuscha* et *O. keta*) et effectué des prises de plancton à l'aide de l'échantillonneur Clarke–Bumpus en trois endroits du rivage de Puget Sound. En 1971, nous avons collectionné des alevins de saumon keta et prélevé des échantillons de benthos à l'aide d'une pompe. Le régime alimentaire est semblable chez les deux espèces. Les proies principales des saumons roses et keta sont des copépodes harpactoïdes épibenthiques (57 et 36%, respectivement, en 1970). Il y a toutefois des différences notables, la plus remarquable étant une préférence, chez les saumons roses, pour les oeufs d'invertébrés et, chez saumons keta, pour les petits amphipodes gammaridés et le harpactoïdes. Le contenu des estomacs n'a aucune ressemblance avec celui des prises de plancton effectuées dans la même région. La phase littorale du développement semble être un stade écologique distinct du cycle biologique de ces espèces.

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THERE has been a significant reduction of pink salmon (*Oncorhynchus gorbuscha*) stocks in Puget Sound in recent years. A trophic analysis was begun to see if a possible food limitation in the initial marine period of growth might be contributing to this, and was to be used in the predictive models of pink and chum (*O. keta*) salmon returns. No published dietary or natural history information exists for juvenile pinks or chums in Puget Sound, the southern portion of their geographic range. Existing literature (Fraser 1946, Neave 1966, Manzer 1969, Merrell 1970, and a series of Russian papers typified by Andrievskaya 1958) suggested that the young of these species would be zooplanktivorous. This study was restricted to fish 6 cm and shorter taken from intertidal waters less than 1.5 m deep. It was designed to compare food items eaten against prey available as sampled by a Clarke–Bumpus net towed in the shallow waters that the fish were inhabiting.

Materials and methods — In 1970 pink and chum fry were collected by beach seine and a long handled dipnet at three areas of Puget Sound from their initial appearance in April until they moved offshore in early June. Sampling was done on six dates at Anderson Island

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(South Puget Sound), on 5 days at Tonandoes Peninsula, Dabob Bay (Hood Canal), and on 4 days at Port Susan (east central). Each site is representative of a major management area of Puget Sound. The beaches are sandy at low tide and are composed of sand, gravel, and cobbles at mid and high tides. The average tidal variation was 3–4 m. A sample of six fish of each species (when available) was arbitrarily selected at each date and immediately fixed with a 10% formalin solution.

The entire digestive tract contents were later examined using a binocular microscope and the prey organisms present were identified, counted, and measured with the aid of a calibrated ocular micrometer.

To estimate prey availability three replicate Clarke–Bumpus zooplankton tows were taken on each date at a fixed depth of 1 m in water about 2 m deep in the area inhabited by the fish. These samples were preserved in 5% formalin and later identified and counted.

In 1971 chum salmon fry were sampled at 10-day intervals. All fish were captured in water less than 1 m deep and within 3 m of shore. The fish were analyzed as in 1970.

Replicated ($n = 3$) "benthic" samples were taken in 1971 with a high-speed water pump (125 liters/min) along a fixed transect approximately 100 m long in each of the three study areas. A 5-cm diameter hose was used with a brass probe at the end to keep the mouth approximately 3 cm above the sediment while constantly agitating the surface of the sediment. The hose mouth was passed in a systematic pattern within a raised screened hoop framing a 0.5 m² area for a fixed duration of 3 min. Pump effluent was retained in a sock-like net (195-μ mesh) 0.3 m long and 5 cm in diameter. The pump sample contents were identified, sized, and enumerated by progressive decantation. The larger samples were split with a Folsom plankton splitter and one-half of the sample was analyzed.

Results — 1970 — Chum Salmon — Fifty-four fish were examined at the three sites. The fish ranged in length from 3.2 to 6.0 cm ($\bar{x} = 4.3 \pm 0.5$ SD) and were always taken from onshore waters. Table 1 lists a summation of the diets of these fish during their onshore residence. The food items are listed in order of their overall mean percent of occurrence. As a generality, the harpacticoid copepods were the chief prey for these juvenile fish in Puget Sound (57% of their diet), followed by small gammarid amphipods (15%) and barnacle nauplii (6%). Some variation in the relative importance of the food items is evident, especially at the Port Susan site. Significant differences in the frequency of occurrence of dietary items do occur at the three sites as determined by Chi Square ($\alpha < 0.001$). The more obvious differences include the striking importance of harpacticoid copepods at Anderson Island and the more catholic feeding at Port Susan.

The coefficients of variation (standard deviations/mean × 100) were determined for the individual study areas as well as for the total feeding pattern for those sample days when the prey item was eaten by at least one fish. A low value indicates a similar feeding pattern amongst fish captured at one sampling, while a high number indicates more variability in feeding. A generally inverse relation was found between the relative importance of a food item and the coefficient of variation on that item.

Pink Salmon — Table 2 lists the prey found in 53 fish taken at the same sites and times as the chum salmon. The pinks ranged from 3.3 to 5.5 cm in length ($\bar{x} = 3.9 \pm 0.5$ SD). There was an overall similarity in feeding between the two species. To test whether significant differences in feeding occurred, the Wilcoxin Signed Rank Test (Steele and Torrie 1960) was used. Pairing (chum vs. pink) on each food item was done on those sampling days when several fish of each species were taken. The chum fry ate significantly more harpacticoid copepods ($\alpha = 0.1$) and adult Diptera ($\alpha = 0.02$), while the pink fry fed more on invertebrate eggs ($\alpha = 0.001$). None of the other prey items occurred in significantly different amounts.

Differences in the proportion of prey items were also found in the pink fry at the three areas ($\alpha < 0.001$), similar in pattern to that found for the chum salmon.

Examination of the plankton samples revealed that the stomach contents bore little resemblance to them. For example, on April 20, the harpacticoid copepods made up 98% of the chums' diet and 95% of the pinks' diet at Anderson Island, while they comprised only 0.4% of the plankton hauls. The chum and pinks ignored the "abundance" of barnacle nauplii on that day (24% of the zooplankton); the nauplii comprised only 0.5% of the pink gut contents and were not taken by the chums. As there was an almost total lack of correspondence between the plankton samples and fish gut contents, the plankton data are not presented.

1971 — Chum — During the spring 78 chum fry were captured and examined. The feeding results were very similar to those obtained for the chum salmon in 1970. The harpacticoids made up 89.1, 83.6, and 7.8% of their diets at Anderson Island, Dabob Bay, and Port Susan, respectively, while the gammarids comprised 0.7, 3.6, and 31% of their diets.

Inspection of the pump samples (taken along the substrate–water interface) revealed that this technique sampled the same group of animals that the fish were feeding on.

TABLE 1. Summation of the diets of juvenile chum salmon (*Oncorhynchus keta*) from three areas of Puget Sound in spring 1970.

	Harpacticoids	Gammarids	Barnacle nauplii	Mysis larvae	Calanoids	Insects (Diptera)	Bivalve larvae	Corycaeus	Insect larvae	Cladocera	Euphausiids	Invertebrate eggs	Calyptopis larvae	Total
Anderson Island ($n = 23$)														
X % of diet	89.0	0.5	0.9	0.8	2.8	0.4	0	3.0	2.0	0	0	0	0.1	99.6
SD	17.9	0.7	1.0	2.0	6.3	0.7	0	10.5	4.8	0	0	0	0.1	
X # prey per fish	193.9	1.2	1.2	0.5	2.0	0.5	0	3.6	1.4	0	0	0	0.1	204.4
SD	154.7	1.7	2.2	1.0	3.5	0.8	0	13.8	3.0	0	0	0	0.2	
X CV of samples	7.3	105.7	100.6	117.4	111.2	133.7	0	67.3	79.1	0	0	0	161.1	85.4
# Samples with item	6	4	4	2	3	4	0	4	2	0	0	0	1	30
Dabob Bay ($n = 21$)														
X % of diet	34.0	19.6	13.5	7.2	8.5	2.5	4.7	2.8	0	0	1.7	0	0.8	95.3
SD	33.8	28.1	24.1	16.4	11.0	6.7	9.9	6.5	0	0	5.2	0	2.4	
X # prey per fish	24.3	24.4	11.0	3.2	5.9	0.5	8.3	3.7	0	0	0.9	0	0.6	82.8
SD	36.5	45.8	23.2	7.7	15.6	0.9	19.6	9.0	0	0	2.0	0	2.0	
X CV of samples	91.0	102.8	152.3	97.2	124.4	183.2	119.2	160.0	0	0	176.1	0	86.8	130.0
# Samples with item	5	5	5	3	5	4	2	2	0	0	3	0	1	35
Port Susan ($n = 10$)														
X % of diet	31.1	36.5	2.9	10.6	0.4	7.7	4.0	0	0	4.4	0	1.8	0	99.4
SD	27.3	28.4	6.9	22.9	1.3	10.0	12.6	0	0	6.3	0	4.3	0	
X # prey per fish	9.6	13.9	0.7	2.4	0.1	3.3	0.9	0	0	1.6	0	0.7	0	33.2
SD	9.0	12.5	1.6	5.3	0.3	5.2	2.8	0	0	2.5	0	1.9	0	
X CV of samples	19.5	34.5	80.0	80.0	325.0	40.4	0	0	0	94.5	0	116.1	0	78.5
# Samples with item	3	2	3	3	1	3	1	0	0	3	0	2	0	20
Total (Weighted) ($n = 54$)														
X % of diet	56.8	14.6	6.2	5.1	4.6	2.6	2.6	2.4	0.9	0.8	0.7	0.3	0.3	97.9
X # prey per fish	93.8	12.6	4.9	1.9	3.1	1.0	3.3	3.0	0.6	0.3	0.4	0.1	0.3	125.3
CV %	39.8	91.4	117.0	95.8	142.2	126.2	79.5	98.2	79.1	94.5	176.1	116.1	123.9	100.9
n	14	11	12	8	9	11	3	6	2	3	3	2	2	

TABLE 2. Summation of the diets of juvenile pink salmon (*O. gorbuscha*) taken from the same areas and times as the chum salmon in spring 1970.

	Harpacticoids	Barnacle nauplii	Invertebrate eggs	Mysis larvae	Calanoids	Cladocera	Gammarids	Bivalve larvae	Corycaeus	Euphausiids	Calyptopis larvae	Insect adults (Diptera)	Insect larvae	Total
Anderson Island ($n = 14$)														
X % of diet	95.9	0.5	0.4	0.3	0.7	0	0.1	0	1.3	0	0+	0	0	99.2
SD	3.9	0.8	1.6	0.7	1.4	0	0.2	0	1.4	0	0.1	0	0	
X # prey per fish	195.9	1.0	0.6	0.6	1.7	0	0.1	0	3.1	0	0.1	0	0	203.1
SD	73.5	1.7	2.4	1.2	2.7	0	0.4	0	3.9	0	0.3	0	0	
X CV of samples	18.8	89.2	175.0	184.7	136.8	0	175.9	0	124.2	0	172.7	0	0	119.8
# Samples with item	4	2	1	3	2	0	2	0	4	0	1	0	0	19
Dabob Bay ($n = 16$)														
X % of diet	27.9	6.4	17.9	3.4	20.0	0	4.1	7.9	4.1	3.9	0.2	0	0	95.8
SD	28.5	6.4	30.0	6.7	21.1	0	8.0	13.6	7.2	8.7	1.0	0	0	
X # prey per fish	17.8	4.0	19.5	0.4	14.8	0	0.8	6.9	2.4	0.7	0.2	0	0	67.5
SD	33.6	6.2	47.0	0.9	28.1	0	1.7	13.2	4.3	1.2	1.0	0	0	
X CV of samples	53.1	86.7	129.5	133.7	80.0	0	178.1	88.0	168.5	149.6	140.0	0	0	112.5
# Samples with item	5	5	3	2	3	0	3	3	3	3	1	0	0	31
Port Susan ($n = 23$)														
X % of diet	5.1	30.9	19.7	21.2	4.6	9.7	6.1	0.6	0.1	0	0.5	0.5	0	99.5
SD	5.6	37.6	31.3	28.5	9.0	16.7	10.8	1.7	0.3	0	3.2	1.3	0	
X # prey per fish	8.3	77.2	18.1	9.3	2.3	8.8	10.6	0.5	0.1	0	0.2	1.7	0	137.1
SD	11.9	128.8	35.6	12.0	3.8	18.5	17.2	1.5	0.3	0	0.8	3.2	0	
X CV of samples	109.7	80.7	88.6	105.8	129.8	104.8	102.1	116.7	86.6	0	200.0	168.6	0	111.3
# Samples with item	5	5	3	4	4	4	4	1	1	0	1	3	0	35
Total (Weighted) ($n = 53$)														
X % of diet	36.0	15.5	14.1	10.3	8.2	4.2	3.9	2.7	1.6	1.2	0.3	0.2	0	98.2
X # prey per fish	60.7	35.0	13.9	4.3	5.9	3.8	4.9	2.3	1.6	0.2	0.2	0.7	0	133.5
CV %	63.5	84.6	118.5	138.3	114.8	104.8	143.8	95.2	136.1	149.6	170.9	168.7	0	113.7
n	14	12	7	9	9	4	9	4	8	3	3	3	0	85

An additional analysis was performed to determine if our sample size was sufficient for describing the diets of these species. Accumulative mean percents of diets for the various prey items were calculated from the chum fry taken from the various locations. At any one location on any one date the feeding patterns were very conservative for the more preferred dietary items but were much more variable for the rarer prey. A series of graphs plotting these cumulative means (for the number of fish analyzed up to six) shows this nicely but this type of analysis is overly expensive to reproduce. Thus, a second technique was developed, one that hopefully reflects the overall power of the study. The dietary items were blocked by sampling dates (5) over the entire season for each location. From each block the gut contents of one fish were chosen at random, a mean value of the percent of each dietary item was derived, and one standard error was calculated (SE = $\sqrt{pq/n}$ because proportionate data is binomial in nature). Then, from the remaining fish, accumulative means and standard errors were derived. Figure 1a shows the results of this analysis for the harpacticoid prey and 1b for the gammarid prey taken by the chum salmon at Anderson Island in 1971. The selection of six fish per sampling date generally allows for the ability to distinguish differences in feeding on the harpacticoids of 16% at the 95% confidence level but only differences of 165% for the gammarids. Lumping the data loses some of the distinctness in feeding pattern.

Discussion — The original purpose of this trophic analysis was to gain an understanding of the factors that might influence the abundance and growth of pink and chum salmon during their estuarian residence in Puget Sound. The relatively large reduction of pink salmon stocks in recent years is reasonably coincidental to the development of the massive salmonid hatchery system in Puget Sound (from 20 million planted fish in the 1950s to over 60 million in 1970, Reed 1971). Parker (1971) concluded that much of the early mortality suffered by juvenile pink salmon in a British Columbia inlet is caused by predation by juvenile coho salmon (*O. kisutch*). This also appears to be true in Puget Sound. Heavy predation by cohos was often observed in our studies. In fact the Washington hatchery system possibly intensifies this situation by the artificial early growth and release of young coho at a time that coincides with the natural migration of the pink and chum fry into salt water. The coho would normally have appeared a month or so after the pinks and chums. Thus, part of the original problem appears to be resolved.

Earlier dietary descriptions (done primarily on older fish in more offshore waters) led us to believe that these young fish would be zooplanktivorous. Our 1970 study design was based on this hypothesis (eg. a comparison of food availability, zooplankton, to actual prey taken). The complete lack of correlation between the plankton samples and the digestive tract contents was surprising. A closer look at the actual prey provided the answer. These juvenile fish were feeding primarily on epibenthic organisms (Fig. 2). The plankton net, fishing at a fixed depth of 1 m in water about 2 m deep, was missing the majority of these organisms. The "benthic" pump technique sampled the same group of animals that the fish fed upon. These latter results

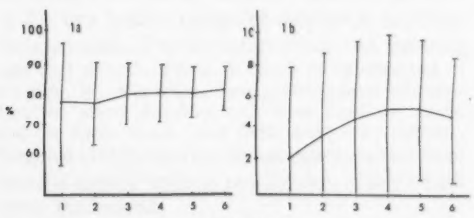

FIG. 1. Accumulative mean percents of diet (and standard errors) as a function of the mean number of juvenile chums (*Oncorhynchus keta*) examined from Anderson Island in 1971. 1a = harpacticoid copepods; 1b = gammarid amphipods.

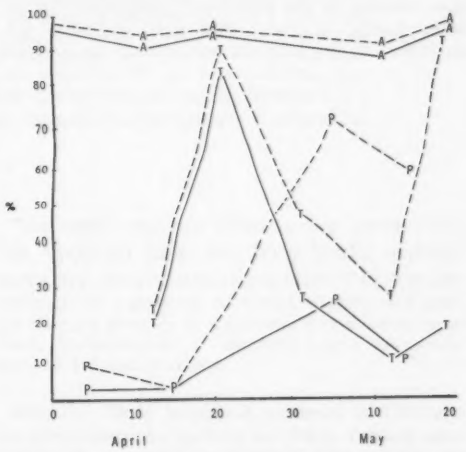

FIG. 2. Importance of harpacticoid copepods (solid lines) and epibenthic organisms (dashes) in the diet of pink (*O. gorbuscha*) and chum fry in various beach areas of Puget Sound in 1970. A = Anderson Island; T = Toandoes Peninsula (Dabob Bay); P = Port Susan.

are being analyzed in more detail to compare availability, electivity, and size selection.

Because the same group of potential prey occurred at the sampling sites on any one day, and the fish were in mixed schools, significant differences in the diets of the two species must represent electivity. Thus, the high incidence of invertebrate eggs in the diets of the pink fry and the higher incidences of adult insect remains and harpacticoid copepods in the chum fry reflect true behavioral preference. Conversely, the significant differences in the proportionalities of the prey items in the three geographical areas probably represent availability, especially since the feeding patterns at each location are somewhat similar for the two species. Figure 2 summarizes these areal differences in the importance of epibenthic prey, especially harpacticoid copepods.

These studies complement that of Healy (1967), who described the shoreline preference exhibited by juvenile pinks in British Columbian waters. LeBrasseur and Parker (1964) quantified this dispersion pattern and showed that it was strongly size specific: "...as fish grow to 4.5 to 5.5 cm they maintain their schooling habit but move farther away from the shoreline...Larger fish are found in the part of the school farthest from the shore and vice versa." Further, they showed that a very dramatic break in the growth curve of pink salmon occurred at about 6–8 cm (from an instantaneous growth rate of 0.559–0.076) and suggested that a definite physiological change occurs at that time "similar to the parr-smolt transformation on other Pacific salmon." Although we have not quantified it, the early growth rate of the juvenile pinks and chums is very rapid in Puget Sound. It appears that the initial marine period in the life cycle of these fish is a distinct ecological stage.

ANDRIEVSKAYA, L. D. 1958. The food of Pacific salmon in the Northwestern Pacific Ocean. Mat. Biol. Morsk. Per. Zhi. Dal. Los., VNIRO, Moscow. 64–75. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 182, 1958.)

FRASER, C. M. 1946. Food of fishes. Trans. Roy. Soc. Can. 40(V): 33–39.

HEALY, M. C. 1967. Orientation of pink salmon (*Oncorhynchus gorbuscha*) during early marine migration from Bella Coola river system. J. Fish. Res. Board Can. 24: 2321–2338.

LEBRASSEUR, R. J., AND R. R. PARKER. 1964. Growth rate of central British Columbia pink salmon (*Oncorhynchus gorbuscha*). J. Fish. Res. Board Can. 21: 1101–1128.

MANZER, J. I. 1969. Stomach contents of juvenile Pacific salmon in Chatham Sound and adjacent waters. J. Fish. Res. Board Can. 26: 2219–2223.

MERRELL, T. R. 1970. Alaska's fishery resources — the chum salmon. U.S. Fish Wildl. Serv. Fish. Leafl. 632: 7 p.

NEAVE, F. 1966. Pink salmon in British Columbia. *In* Salmon of the North Pacific Ocean — Part III, a review of the life history of North Pacific salmon. Int. N. Pac. Fish. Comm. Bull. 18: 73.

PARKER, R. R. 1971. Size selective predation among juvenile salmonid fishes in a British Columbia inlet. J. Fish. Res. Board Can. 28. 1503–1510:

REED, D. [ed.] 1971. State of Washington Department of Fisheries 1970 Annual Report. Wash. Dep. Fish. 80: 159 p.

Age, Growth, and Maturity of Bottlenosed Dolphin (*Tursiops truncatus*) from Northeast Florida

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SERGEANT, D. E., D. K. CALDWELL, AND M. C. CALDWELL. 1973. Age, growth, and maturity of bottlenosed dolphin (*Tursiops truncatus*) from northeast Florida. J. Fish. Res. Board Can. 30: 1009–1011.

It is confirmed that one growth layer consisting of one opaque and one translucent zone is laid down annually in the dentine of the teeth of bottlenosed dolphin (*Tursiops truncatus*), allowing absolute age determination, although the seasonal sequence of dentine deposition is not yet clear. On this basis females from northeast Florida were found to mature at about 12 years and males at 13 years, both sexes living to about 25 years of age. Females accumulated up to 14 corpora albicantia in the ovaries, indicating an accumulation rate of about one per annum. Birth occurred at 100-cm length. Males and females matured at about 245- and 235-cm length, respectively, and attained asymptotic lengths of about 270 and 250 cm.

SERGEANT, D. E., D. K. CALDWELL, AND M. C. CALDWELL. 1973. Age, growth, and maturity of bottlenosed dolphin (*Tursiops truncatus*) from northeast Florida. J. Fish. Res. Board Can. 30: 1009–1011.

Les auteurs confirment la formation d'une couche annuelle de croissance consistant en une zone opaque et une zone translucide dans la dentine des dents de *Tursiops truncatus*. Grâce à cette couche, on peut déterminer l'âge absolu des individus, bien que la séquence saisonnière de la formation de la dentine ne soit pas encore élucidée. On a déterminé de cette façon que les femelles du nord-est de la Floride atteignent la maturité sexuelle à l'âge d'environ 12 ans, et les mâles à 13 ans, les deux sexes ayant une durée de vie d'environ 25 ans. Les femelles accumulent jusqu'à 14 corpora albicantia dans leurs ovaires, ce qui indique un taux d'accumulation d'environ un par année. Les jeunes mesurent 100 cm à la naissance. Les mâles atteignent la maturité sexuelle à une longueur d'environ 245 cm, les femelles à 235 cm. La courbe de croissance des mâles atteint son assymptote à une longueur d'environ 270 cm, celle des femelles à environ 250 cm.

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ALTHOUGH bottlenosed dolphin (*Tursiops truncatus*) is the best known species of dolphin in captivity, little is known of its life history in the wild, including age and growth. The collections of Marineland of Florida, St. Augustine, have given unique information on many dolphins that have lived for years, and in some cases have been born, in captivity. Sergeant (1959) reported on age determination from dentinal growth layers in two *Tursiops* of known age from Marineland.

Detailed studies on reproduction of *T. truncatus* reported by Harrison et al. (1969) and Harrison and Ridgway (1971) suffered from lack of knowledge of age of animals studied.

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The earlier data are added to new material for this report on teeth, sex, body length, maturity status, and dates of capture and death of 62 dolphins collected in waters off northeast Florida and held for varying periods in captivity. Three were born there. Examination of dentinal layers has again allowed estimation of age.

Methods — Body length was measured in a straight line from nosetip to notch of tail flukes. Females were categorized as mature or immature from presence or absence of corpora lutea and corpora albicantia, which were also counted. Males were described as immature, maturing, or mature from presence or absence of sperm and size of testes. (A detailed histological study of the gonads is in preparation.) To estimate age, we examined either the surface of sagittally sectioned half-teeth or thin

longitudinal sagittal slices (Fig. 1), then counted tooth layers by eye. Additional criteria used in checking the relative accuracy of age determination were the wear on the tip of the teeth (heavy in one old specimen) and the degree of closure of the pulp cavity, which is progressive.

Age — Counts of tooth layers are given (Table 1) for three newly-reported and the two previously-reported known-age animals and for the animals that had lived more than 4 years in captivity. The only good direct evidence on the frequency of tooth layering is given by the tooth of No. 7 ("Spray") in which, however, the layers were faint and the numbers of layers not easy to read. Hence, the group of wild-caught animals furnish important additional evidence. Since the number of layers was in all cases less than twice the known years in captivity, there must be less than two layers laid down per annum and, since the number must be an integer, it is one.

These results are consistent with Sergeant's (1959) hypothesis that one growth layer, consisting of one opaque and one translucent zone, is laid down each year in *T. truncatus*. An attempt to determine at what seasons the two types of dentine growth were laid down was not successful; larger material, especially of young animals, is required for such an analysis.

In teeth of the 21-year-old Spray, a lifelong captive, the layers were fainter than in other porpoises. Therefore the cycle, whatever its nature, leading to the alternation of the two types of dentine deposition is reduced in intensity, although not eliminated, in captivity.

Apart from Spray, the greatest ages read for wild-caught animals were 25 years for two males, and 24 years for a female that had heavily worn tooth-crowns.

TABLE 1. Ages and numbers of dentine layers for bottlenosed dolphins (*Tursiops truncatus*) born in captivity at Marineland, or caught in wild but living in captivity for more than 4 years.

Born in captivity		Born in wild	
Age (*years*)	No. of dentine layers	Years in captivity	No. of dentine layers
0.2	<0.5	4.3	8
0.5[a]	<1	5.8	10
1.25	1.25	7.8	14
2.4[a]	2.4	8.0	10
21.0	≃21	10.3	11.5
		10.6	12
		10.6	18
		11.1	12
		11.5	ca. 17
		12.4	18

[a]Data from Sergeant (1959).

Sex ratio — Of the 62 animals, one was not ageable and one lacked data on length, sex, and maturity status. Of 61 animals, 30 were females and 31 males, giving a sex ratio not significantly different from unity. True (1890) found 82 ♂♂ and 75 ♀♀ at Cape Hatteras. This result, together with the finding that greatest ages for the two sexes are similar, suggests that their mortality rates are about the same.

Dr W. L. van Utrecht, Amsterdam, is making independent age determinations of this tooth material using a photoelectric scanner and plotter. He notes (in litt., May 4, 1971) that the teeth show the consistent sexual difference found by him (Utrecht 1969) in teeth of *Phocoena phocoena*, namely a nonoverlapping longer primary (neonatal) line, as viewed in longitudinal section, in males than in females. This feature allows sex determination from skulls of animals the sex of which is not otherwise known.

Growth and maturity — The males attained an asymptotic length of some 270 cm as compared with 250 cm for females (Fig. 2).

Males attained sexual maturity at a length of about 245 cm and females at 235 cm, or about 90% of asymptotic growth. Immature males had testes weighing 10–15 g; a class of maturing bulls, 100–200 g; and mature bulls, 300–700 g (Fig. 3a). Maturing males occurred between 7 and 15 years of age, and mature bulls from 12 years and up (Fig. 3b).

Females matured at about 12 years of age (Fig. 2), or slightly younger than males. Since 14 corpora albicantia may accumulate between the ages of 12 and 24 years (Fig. 4), the mean accumulation rate is approximately one per annum. The exception to these statements is the captive Spray, which is known to have matured at 6 years since it produced a first calf at 7 years (Hill 1956) and gestation lasts 12 months in *T. truncatus* (McBride and Kritzler 1951). Moreover, in 15 years of maturity it only produced seven corpora albicantia. It is therefore necessary to assume that this animal matured early under captive life, but had fewer opportunities than in nature for reproduction.

Harrison et al. (1969), on the contrary, suggest that porpoises in captivity may have less opportunity

FIG. 1. Longitudinal slices of undecalcified bottlenosed dolphin (*Tursiops truncatus*) teeth cut with Gillings-Hamco thin sectioning machine (Hamco Machines Inc., Rochester, N.Y.) at 80 μ. (a) #211, ♀, 165 cm, known age 1.25 years; (b) #181, ♀, 202 cm, 5 years; (c) #7, ♀, 250 cm, 21 years in captivity; (d) #R8, ♂, 285 cm, 25 years.

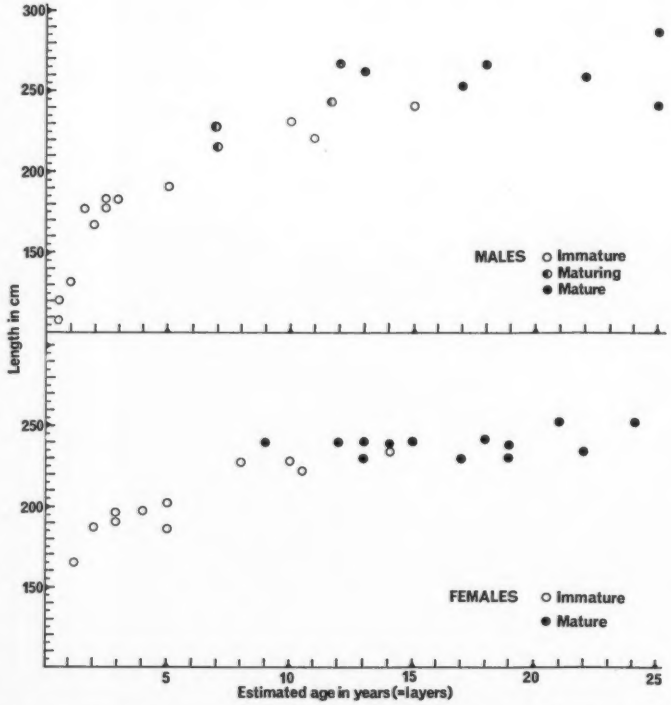

FIG. 2. Growth curve and maturity status for male and female *Tursiops truncatus* from northeast Florida.

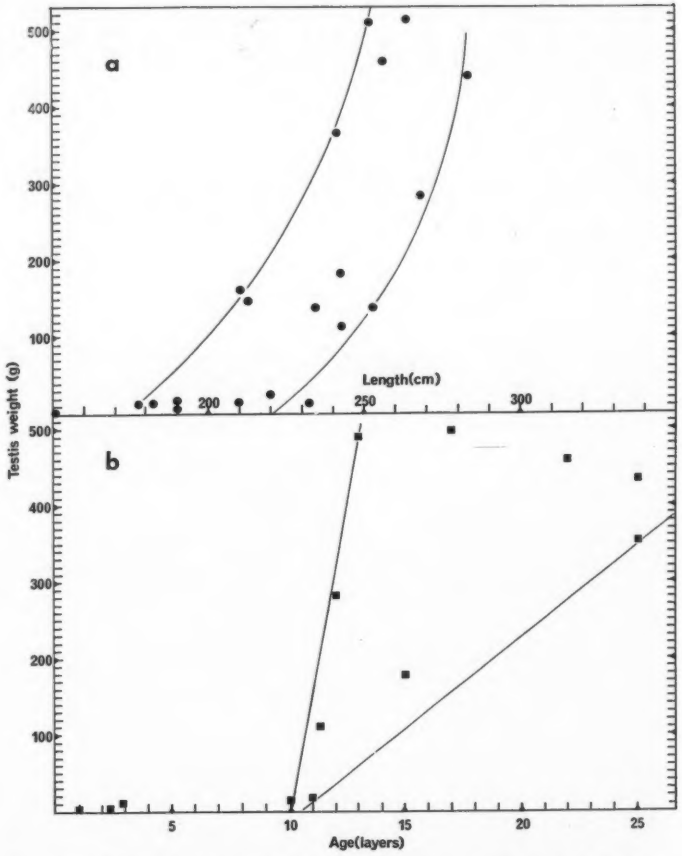

FIG. 3. *Tursiops truncatus* males. Testis weight plotted against a, length; b, age.

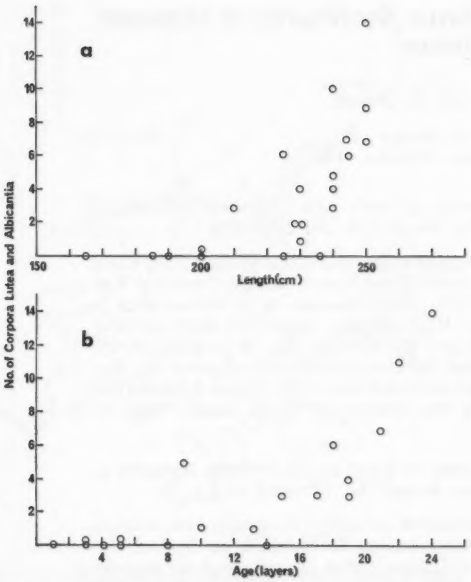

FIG. 4. *Tursiops truncatus* females. Number of corpora lutea plotted against a, length; b, age.

to mate so that sexual maturity is delayed. We have, therefore, separated the available sample into two parts, consisting of animals that have lived less and more than 1 year in captivity. There is no suggestion from the analysis (Table 2) that captive males showed delayed maturity, and only a slight suggestion that the females were so delayed. There seems no reason to suppose that sexual maturation depends on opportunity to mate, but rather that captive life with improved nutrition produces improved growth and maturation rates.

Discussion — *Tursiops truncatus*, a generalized delphinid species in body form and color pattern (Mitchell 1970), also shows generalized characters of life history. There is little sexual dimorphism (confirming True 1889) in age at maturity, in longevity, and, therefore, of mortality rate. Similar features are found for the more specialized, long-snouted species *Delphinus delphis* (Sleptsov 1941). In the long-snouted species *Stenella caeruleoalba*, Kasuya (1972), using dentinal growth layers, found sexual maturity attained at 9 years in both sexes, a result very similar to the present one. However, in the specialized short-snouted species *Globicephala melaena* (Sergeant 1962) the male is much larger and matures much later than the female, the sex ratio shows

TABLE 2. Ages at sexual maturity in the wild and in captivity. Wild animals held for 1 year or less, and captive animals for more than 1 year.

Category		Youngest mature	Oldest immature	Mean
Captive	F	10, 12	14	12
	M	11½	10	11
Wild	F	10½	9	10
	M	7	11, 15	11

more females, and there is a high ratio of adult females to adult males. These adaptations are believed to lower the degree of fighting between males and, consequently, improve the social cohesion of the herd. Since dominant male *T. truncatus* become aggressive to other males in the spring sexual season, it seems likely that at this time herds of subordinate males will be found, as reported to, but not seen by, True (1890).

HARRISON, R. J., R. C. BOICE, AND R. L. BROWNELL. 1969. Reproduction in wild and captive dolphins. Nature (London) 222: 1143–1147.

HARRISON, R. J., AND S. H. RIDGWAY. 1971. Gonadal activity in some bottlenose dolphins (*Tursiops truncatus*). J. Zool. (London) 165: 355–366.

HILL, R. N. 1956. Window in the sea. Rinehart, New York, N.Y. 208 p.

KASUYA, T. 1972. Growth and reproduction of *Stenella caeruleoalba* based on the age determination by means of dentinal growth layers. Sci. Rep. Whales Res. Inst. 24: 57–79.

MCBRIDE, A. F., AND H. KRITZLER. 1951. Observations on pregnancy, parturition and postnatal behavior in the bottlenose dolphin. J. Mammalogy 32: 251–266.

MITCHELL, E. D. 1970. Pigmentation pattern evolution in delphinid cetaceans: an essay in adaptive coloration. Can. J. Zool. 48: 717–740.

SERGEANT, D. E. 1959. Age determination of Odontocete whales from dentinal growth layers. Norw. Whaling Gaz. 1959: 273–288.

1962. The biology of the pilot or pothead whale *Globicephala melaena* in Newfoundland waters. Fish. Res. Board Can. Bull. 132: 84 p.

SLEPTSOV, M. M. 1941. O biologii razmnodjeniya chernomorskogo delfina *Delphinus delphis*. (On the reproduction of Black Sea *Delphinus*.) Zool. Zh. 20: 632–653.

TRUE, F. W. 1889. A review of the family Delphinidae. Bull. U.S. Mus. Natur. Hist. 36: 191 p.

1890. Observations on the life history of the bottlenose porpoise. Proc. U.S. Nat. Mus. 13: 197–203.

UTRECHT, W. L. VAN. 1969. A remarkable feature in the dentine of Odontocetes. Beaufortia 16: 157–162.

Homing of Brook Trout (*Salvelinus fontinalis*) in Matamek Lake, Quebec[1]

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O'CONNOR, J. F., AND G. POWER. 1973. Homing of brook trout (*Salvelinus fontinalis*) in Matamek Lake, Quebec. J. Fish. Res. Board Can. 30: 1012–1014.

Brook trout (*Salvelinus fontinalis*) showed both in-season and repeat homing to spawning tributaries of Matamek Lake, Quebec. Of 283 trout clipped from Gallienne Creek and Kaikhosru Creek during August and September, 1971, 31.1% returned to the homestreams to spawn in 1972. During July and August, 1972, 450 trout were tagged and displaced from these two streams and released at three points (0.20–4.50 km) in the lake. Of these fish, 44.0% homed, 0.2% strayed to the opposite stream and the remainder were unaccounted for. Percentage returns from the deepwater release point was much lower than returns from the two inshore release points, and average return time was proportional to the distance from the homestream.

O'CONNOR, J. F., AND G. POWER. 1973. Homing of brook trout (*Salvelinus fontinalis*) in Matamek Lake, Quebec. J. Fish. Res. Board Can. 30: 1012–1014.

L'omble de fontaine (*Salvelinus fontinalis*) démontre un instinct de homing, tant au cours de la saison qu'au temps de la fraie, dans les tributaires du lac Matamek, au Québec. Des 283 ombles de fontaine des ruisseaux Gallienne et Kaikhosru dont on avait coupé une nageoire en août et septembre 1971, 31.1% sont retournés frayer dans leur cours d'eau d'origine en septembre 1972. Au cours de juillet et août 1972, 450 ombles de fontaine ont été étiquettés et transportés de ces deux ruisseaux au lac Matamek, où ils ont été libérés à trois stations différentes (0.20–4.50 km). De ces poissons, 44.0% sont revenus à leur ruisseau d'origine, 0.2% au ruisseau opposé, et on ignore le sort du reste. Le pourcentage de retours d'ombles de fontaine libérés en eau profonde est beaucoup plus bas que celui des poissons libérés aux deux stations du littoral; de plus, le temps moyen du retour est proportionnel à la distance du ruisseau d'origine.

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A number of homing studies have been carried out on salmonids. Stuart (1957) has shown in-season and repeat homing for brown trout (*Salmo trutta*). Ball (1955) found that adult cutthroat trout (*Salmo clarki*) returned to their home streams and this characteristic was also reported by Lindsey et al. (1959) for adult rainbow trout and by Shapovalov and Taft (1954) for steelhead trout (*Salmo gairdneri gairdneri*) and coho salmon (*Oncorhynchus kisutch*).

Surprisingly little work has been done on the homing ability of brook trout (*Salvelinus fontinalis*). Vladykov (1942) carried out repeat and in-season homing studies in Lac Epaule in Laurentides Park, Quebec. The returns for these two studies were <1.0% and 8.8%, respectively. These results did not indicate conclusively that brook trout have a homing tendency. Therefore, it was hoped that a more intensive study would resolve the question.

Materials and methods — During the summers of 1971 and 1972 we conducted in-season and repeat homing experiments on brook trout originating from and spawning in tributaries of Matamek Lake. This lake (Fig. 1) has an area of 1620 ha and a maximum depth of 100 m. It has an elevation of 122 m and is located 35 km northeast of Sept Iles, Quebec.

Between August 16 and 22, 1971, 129 migrants were trapped at the mouth of Gallienne Creek (Fig. 1), and marked with a pelvic fin clip. On September 5 and 6, 154 fish were seined on their spawning grounds in Kaikhosru Creek over 1250 m upstream from the mouth. These fish were marked with an adipose fin clip and lengths were recorded.

In 1972, trapnets were placed across Gallienne Creek and Kaikhosru Creek at distances of 150 m and 1250 m, respectively, from the stream mouths. From July 26 to August 13, fish were removed from the traps daily and placed in plastic cans containing 25–30 liters of fresh water. The fish were then carried back to the lake,

[1]Contribution No. 2991 from the Woods Hole Oceanographic Institution, Woods Hole, Mass. 02543, USA.

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FIG. 1. Matamek Lake, showing lower reaches of study streams and points where brook trout (*Salvelinus fontinalis*) were released.

anesthetized with MS222, tagged below the dorsal fin with a modified Carlin tag, and had lengths recorded. Fish that appeared in good condition after recovering from the anesthetic were released at three points in the lake (Fig. 1). Distances (kilometers) from the release points to the stream mouths are as follows:

	A	B	C
Gallienne	0.20	2.65	4.50
Kaikhosru	0.20	2.25	4.25

Seventy-five tagged fish from each stream were released at each point for a total of 450 displaced trout. The traps were operated continuously during the spawning run until September 6 when severe floods washed them out. Tag number and recapture dates were recorded for all returning fish.

Results — Sixty-six (42.8%) of the fish marked in Kaikhosru Creek in 1971 returned to the stream to spawn in 1972. The number of recaptures in Gallienne Creek was lower with 22 (17.0%) of the clipped fish returning. Of the 225 fish tagged in Kaikhosru Creek in 1972, 121 (53.3%) homed and 1 (0.4%) strayed within season, whereas in Gallienne Creek, of the 225 fish tagged, 77 (34.2%) homed and no strays were recorded. The percentage returns from release point B were lower than returns from A or C and the average return time was proportional to the distance from the home stream (Table 1). Students *t*-test (Steel and Torrie 1960) showed that the differences in return numbers and return times were not significant at the 5% level of probability.

Some in-season returns of stream-resident fish that had migrated from Kaikhosru Creek returned to the same stream to spawn. One fish that was a resident of Sherry Creek (a study stream on the south end of the lake) in 1972 migrated to the lake and moved up Kaikhosru Creek to spawn.

Discussion — The most striking feature was the high proportion of displaced trout that returned to their home stream and the accuracy with which they discriminated between Kaikhosru Creek and Gallienne Creek. Only one out of 198 fish recaptured had moved sufficient distance up the wrong stream to enter the trap. This is all the more remarkable when one considers the proximity of the two stream mouths and the similar terrain traversed by the streams. Obviously homing is not perfect as the one fish from Sherry that moved into Kaikhosru indicates, but compared to some in-season homing experiments done on cutthroat trout in Yellowstone Lake the brook trout shows little evidence of straying. Jahn (1969) reported that 7.5% and 15.0% strayed while McCleave (1967) found that 15.6% of his trout strayed. Both these authors related the high number of strays to a phenomenon called proving, in which a fish may enter any stream before rejecting it and finally moving up its home stream.

There was a much higher percentage return of brook trout releases from A and C which were both inshore release points as compared to point

TABLE 1. Results of marking and displacement of brook trout (*Salvelinus fontinalis*) migrating up Kaikhosru and Gallienne, tributaries of Matamek Lake. Seventy-five fish were marked and released at each location for each tributary. Ranges in parentheses.

	Release point		
	A	B	C
	% recaptured		
Kaikhosru	64.0	41.3	56.0
Gallienne	40.0	22.7	40.0
Average	52.0	32.0	48.0
	Days after release		
Kaikhosru	12.6 (2–30)	16.8 (5–32)	19.0 (3–32)
Gallienne	10.0 (2–27)	10.2 (5–38)	12.6 (4–40)
Average	11.3	13.5	15.8

B which was well offshore where the depth was over 100 m. Jahn (1966) suggested that fish in shallow water might get cues for directing them toward their spawning stream. Some of the fish released at point B may have been delayed in the deeper water before finding the shallow water and their way back to the home stream.

A small hole was discovered in the trapnet in Gallienne during the peak of the spawning run on August 31. This may have existed for 2 or 3 days and was probably the principal reason for the difference in recaptures of both repeat and in-season spawners between the two streams.

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BALL, O. P. 1955. Some aspects of homing in cutthroat trout. Proc. Utah Acad. Sci. Arts Lett. 32: 75–80.

JAHN, L. A. 1966. Open-water movements of the cutthroat trout (*Salmo clarki*) in Yellowstone Lake after displacement from spawning streams. J. Fish. Res. Board Can. 23: 1475–1485.

1969. Movements and homing of cutthroat trout (*Salmo clarki*) from open water areas of Yellowstone Lake. J. Fish. Res. Board Can. 26: 1243–1261.

LINDSEY, C. C., T. G. NORTHCOTE, AND G. F. HARTMAN. 1959. Homing of rainbow trout to inlet and outlet spawning streams at Loon Lake, British Columbia. J. Fish. Res. Board Can. 16: 695–719.

MCCLEAVE, J. D. 1967. Homing and orientation of cutthroat trout (*Salmo clarki*) in Yellowstone Lake, with special reference to olfaction and vision. J. Fish. Res. Board Can. 24: 2011–2044.

SHAPOVALOV, L., AND A. C. TAFT. 1954. The life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*). Calif. Dep. Fish Game Fish Bull. 98: 375 p.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics, with special reference to the biological sciences. McGraw-Hill, New York, N.Y. 481 p.

STUART, T. A. 1957. The migration and homing behaviour of brown trout (*Salmo trutta* L.). Scot. Home Dep. Freshwater Salmon Fish. Res. 18: 1–27.

VLADYKOV, V. D. 1942. Precision with which speckled trout (*Salvelinus fontinalis*) return to the same spawning grounds. Can. Field Natur. 56: 134–136.

Preliminary Survey of Mercury and Other Metals Contained in Animals from the Fraser River Mudflats

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PARSONS, T. R., C. A. BAWDEN, AND W. A. HEATH. 1973. Preliminary survey of mercury and other metals contained in animals from the Fraser River mudflats. J. Fish. Res. Board Can. 30: 1014–1016.

Benthic animals from Sturgeon Bank contained larger amounts of mercury and other metals than animals from similar environments on the coast of British Columbia. These high levels of metals are believed to be associated with the Vancouver City sewer outfall.

PARSONS, T. R., C. A. BAWDEN, AND W. A. HEATH. 1973. Preliminary survey of mercury and other metals contained in animals from the Fraser River mudflats. J. Fish. Res. Board Can. 30: 1014–1016.

Les animaux benthiques du banc Sturgeon contiennent de plus fortes quantités de mercure et autres métaux que ceux d'habitats semblables du long de la côte de la Colombie-Britannique. On croit que l'émissaire d'égouts de la ville de Vancouver est responsable de ces hautes concentrations de métaux.

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A survey of the benthic animal community of the Fraser River mudflats (49°10′N, 123°15′W) was carried out during the summer of 1972. The purpose of this study was to describe the principal species making up the community and to determine the level of pollutants contained in the macrofauna. Since there is currently a great deal of interest in the future development of this area it would appear that our results on heavy metal analyses may be of assistance to various decision making groups. In particular the attention which has recently been focused on the mercury content of dogfish in the area of the Fraser estuary, raises a question as to the source of this metal. It has been suggested that "contamination by effluents from industrial establishments in various places on the Fraser River and estuary may be a contributing factor" (Forrester et al. 1972). The following results indicate a more specific origin of this form of pollution.

Materials and methods — For the purposes of comparison, the Fraser River mudflat community was divided into two areas. These were Sturgeon Bank and Roberts Bank which lie north and south of the main arm of the Fraser, respectively. For further comparison, crabs and other marine organisms were also collected from the Cowichan River mudflats (48°45′N, 123°37′W) and from Kitimat Arm (53°50′N, 128°50′W). All animals were dissected to remove soft tissue, the samples were freeze-dried and powdered to pass a #80 mesh sieve. A 0.25–0.6 g sample of dry powdered tissue was digested with concentrated nitric acid, evaporated to dryness, and the residue taken up in 20 ml of 1.5 M hydrochloric acid. The solutions were then analysed by atomic absorption spectrophotometry (Techron AA-4 and Perkin Elmer Model 303) for Ag, Cd, Co, Cu, Mn, Ni, and Zn; Pb was determined separately by colorimetric analysis. For Hg analysis samples were digested with H_2SO_4 and H_2O_2; Hg was analysed in a flameless cell on a Jarell–Ash atomic absorption spectrophotometer. All analyses are reported in ppm on a dry weight basis; for conversion of these results to wet weight values, they should be divided by 5 for crabs and 20 for molluscs.

Results — The mercury content of crabs taken from the two areas of the Fraser River mudflats, Cowichan Bay, and Kitimat Arm is shown in Table 1. The significant feature of these analyses is that the larger crabs taken from Sturgeon Bank contained approximately seven times the amount of mercury compared with the same sized animals from Roberts Bank, the Cowichan River estuary, or Kitimat Arm. According to Butler (1961), crabs having a carapace width of >110 mm would be 3–5 years old; crabs of less than approximately 110 mm carapace width would be in their first or second year. The mercury content of crabs from Sturgeon Bank which were less than 2 years old is much lower than animals 3–5 years old. This may be due to a change in feeding regime (T. H. Butler personal communication) in which older crabs feed on many types of food, including debris, while younger crabs are more specific in their food requirements.

Molluscs collected from Sturgeon Bank, Roberts Bank, and the Cowichan River estuary were generally too small to analyse as individuals, but five

TABLE 1. Mercury content of crab muscle (*Cancer magister* unless otherwise noted).

Sturgeon Bank		Roberts Bank		Cowichan Bay		Kitimat Arm	
Size[a] (*mm*)	Hg (*ppm*)	Size[a] (*mm*)	Hg (*ppm*)	Size[a] (*mm*)	Hg (*ppm*)	Size[a] (*mm*)	Hg (*ppm*)
95	0.48	119[b]	0.31	135[b]	0.34	121	0.14
98	0.43	122	0.21	145	0.12	142	0.42
99	0.37	125	0.18	151	0.44	144	0.49
108	0.43	137[b]	0.25	154[b]	0.14	156	0.26
147	3.7	138	0.34	160	0.13	170	0.50
148	2.2	146	0.32	165	0.25		
151	3.0	155	0.90	165	0.15		
152	1.9						
152	1.5						
159	3.4						
161	1.5						
161	2.0						

[a]Width of carapace.
[b]*Cancer productus.*

separate collections of animals from Sturgeon Bank gave a mean mercury content of 0.33 ppm (SD 0.08) while five collections from Roberts Bank and two collections from the Cowichan River estuary gave a mean mercury content of 0.12 ppm (SD 0.03). While these figures represent a grouping of data regardless of species, a comparison of the same species (eg. *Crassostrea gigas, Mya arenaria, Mytilus edulis*) all showed higher mercury contents in the Sturgeon Bank specimens. Thus, apparently the higher mercury levels observed in crabs from Sturgeon Bank were also found in the mollusc community of the same area.

Of the other metals analysed, the highest levels were nearly always encountered in animals from Sturgeon Bank. The level of silver, for example, in all 53 animal collections analysed was less than 2 ppm, except in five animals from Sturgeon Bank. In these, levels of >5 ppm were encountered, including one oyster (*Crassostrea gigas*) containing 29 ppm and a group of clams (*Mya arenaria*) containing 17 ppm of silver. Cadmium was less than 2 ppm in all samples except four from Sturgeon Bank and two from Roberts Bank, including one ghost shrimp (*Callianassa californiensis*) which contained 7 ppm. Copper was highly variable, depending on the species of invertebrate analysed. The range of values for Sturgeon Bank crabs was 38–150 ppm compared with a range of 22–68 ppm for all other areas. Co, Mn, Ni, Pb, and Zn did not show any specific pattern of high or low values which could be related to geographical differences in the animals' environment. Full details of all analyses and a description of the mudflat community will be made available in the near future as a data report to be issued by the Westwater Research Centre, University of British Columbia, B.C.

From the results given above it is apparent that, for the most part, the highest concentrations of mercury, and some other metals, were found in animals from Sturgeon Bank. This indicates that the Fraser River itself is probably not the source of high heavy metal concentrations since one would then expect to find similar values on Roberts Bank. One obvious difference between these two adjacent areas is that the Vancouver City sewer outfall empties onto Sturgeon Bank while Roberts Bank is free of any large outfall. In this event one might speculate that the source of heavy metal contamination in the outfall could come from the very widespread use of heavy metals by the citizens of Vancouver (eg. in paints, pharmaceuticals, photographic reagents, etc.) and the ultimate disposal of some fraction of these materials through the sewage system. In this event, an inventory of heavy metal use and disposal in the Greater Vancouver area would be useful information for future planners.

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BUTLER, T. H. 1961. Growth and age determination of Pacific edible crab *Cancer magister* Dana. J. Fish. Res. Board Can. 18: 873–891.

FORRESTER, C. R., K. S. KETCHEN, AND C. C. WONG. 1972. Mercury content of spiny dogfish (*Squalus acanthias*) in the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 29: 1487–1490.

Observed Liver Changes in Rainbow Trout (*Salmo gairdneri*) Fed Varying Levels of a Casein–Gelatin Mixture and Herring Oil in Experimental Diets[1]

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LEE, D. J., AND J. H. WALES. 1973. Observed liver changes in rainbow trout (*Salmo gairdneri*) fed varying levels of a casein-gelatin mixture and herring oil in experimental diets. J. Fish. Res. Board Can. 30: 1017–1020.

Young rainbow trout (*Salmo gairdneri*) were fed semipurified diets containing varying levels of herring oil, casein-gelatin (70–30), and starch for 18 weeks. Fish fed diets containing 53% protein and fat levels of 8 and 16% developed livers with abnormal nuclei, bile duct proliferation, and some parenchymal cell injury. The same level of protein with 24% herring oil did not produce these liver changes. Increasing protein levels with concurrent lowering of starch content produced large livers that were high in glycogen. This effect was not as pronounced at the high level (24%) of fat. The liver abnormalities apparently did not impair growth since weight gains were unrelated to the condition of the liver histology.

LEE, D. J., AND J. H. WALES. 1973. Observed liver changes in rainbow trout (*Salmo gairdneri*) fed varying levels of a casein-gelatin mixture and herring oil in experimental diets. J. Fish. Res. Board Can. 30: 1017–1020.

Nous avons soumis de jeunes truites arc-en-ciel (*Salmo gairdneri*) à des régimes semi-purifiés contenant diverses concentrations d'huile de hareng, de caséine-gélatine (70–30) et d'amidon, pendant 18 semaines. Les poissons nourris de régimes contenant 53% de protéine et des teneurs en graisse de 8 et 16% développent un foie avec noyaux anormaux, une prolifération du canal biliaire et des dommages aux cellules parenchymales. Le même niveau de protéine avec une concentration de 24% d'huile de hareng ne cause pas ces changements au foie. Une augmentation du niveau protéique, accompagnée d'une diminution du contenu en amidon, produit une hypertrophie du foie avec haute teneur en glycogène. Cet effet n'est pas aussi prononcé au niveau élevé (24%) de gras. Ces anomalies du foie ne semblent pas nuire à la croissance, puisque les grains pondéraux n'ont pas de rapport avec la condition histologique du foie.

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THE use of casein as a protein source in experimental diets is an established practice among workers conducting experiments with both poikilothermic and homeothermic species. Casein-gelatin mixtures have been successfully used in test diets for fish by several workers (Halver 1957; Halver et al. 1957; DeLong et al. 1958; Lee et al. 1967; Castell et al. 1972). In most nutritional studies growth is the primary criterion used to evaluate diets and little attention is given to dietary effects on histology. This note describes unusual liver changes that were observed in trout fed varying levels of casein-gelatin (70–30), herring oil, and corn starch.

Materials and methods—Rainbow trout (*Salmo gairdneri*) of the Mt. Shasta strain were divided into 27 lots of 60 fish each. All lot weights were adjusted to within 4 g at the start of the experiment. The fish, 4.5 g at the beginning of the experiment, had been fed a modification of the test diet described by Lee et al. (1967). The original 10% corn oil was replaced with 10% salmon oil, Bernhart–Tomarelli salt mix was used instead of USP XIII, and the vitamin A content was lowered to 25,000 IU/kg. The fish were held in 3- and 4-ft circular fiberglass tanks with a flow of 5 gal of 12.2 C water/min. During the 18-week feeding trial triplicate lots received one of the nine test diets shown in Table 1. Livers from 6 fish/lot were taken for analysis when the experiment was terminated.

Fat and glycogen levels in livers were determined on pooled samples from each lot. Fat was determined by homogenizing the livers in a Virtis mixer with three volumes of water (v/v) and extracting the homogenate three times with two volumes of chloroform/methanol

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aqueous phase was treated with 10% trichloroacetic acid and centrifuged to remove the protein and nucleic acids. Carbohydrate content was determined by analyzing duplicate aliquots of the supernatant with the Anthrone test (Carroll et al. 1956).

Liver samples for histological examination were taken from five of the six livers used for lipid and glycogen analysis. A small slice of tissue was fixed in Bouin's fluid, embedded in paraffin, and stained with hematoxylin and eosin. The prepared tissue was examined for glycogen content and scored 1, 2, 3 for low, medium, or high glycogen content, respectively. The score was determined by noting the relative area of liver section that was vacuolated, assuming that the vacuoles were originally filled with glycogen. We had previously determined by the PAS technique that vacuoles of the type being evaluated in this study contained glycogen rather than fat.

The extent of abnormalities in the size and shape of parenchymal cell nuclei were rated 0 (normal), 2, or 3. The amount of bile-duct proliferation observed was graded 0 (normal) through 5. Each section was examined for abnormal intracellular structure, deposits of cellular debris, and other indications of injury to the parenchymal cells.

Results — The average weights, liver sizes (as grams/100-grams body weight), liver lipid and glycogen levels, and the results of the histological examination are reported in Table 2.

Diets 1 and 4 ,which contained 36% casein-gelatin mixture and 8 and 16% lipid, respectively, were the only diets to produce significantly slower growth rates ($P < 0.05$). However, considerable differences in feed conversion were found. Gain/feed values were 0.74, 0.90, 1.00, 0.81, 0.98, 1.09, 0.96, 1.07, and 1.16 for diets 1 through 9, respectively. Diet 2 (44% protein mix and 8% lipid) produced intermediate gains.

Liver size, in relation to body weight, was directly associated with dietary protein. At each energy level increasing dietary protein levels increased liver size. The percent of total dietary calories derived from protein had a marked effect on liver lipid levels. Diets 2 and 6, 4 and 8, and 1, 5, and 9 produced liver lipid percentages of 1.7 and 1.6, 1.9 and 1.8, and 2.3, 2.3, and 2.0, respectively.

Liver glycogen was positively correlated with dietary protein ($P < 0.02$). Highest liver glycogen levels were found in fish fed 53% protein mix and 8% lipid. In general, there was good agreement between chemically determined levels of glycogen and those derived from histological examination.

The only lots of fish that showed parenchymal cell injury were ones fed diets 3 and 6. These two lots also had a considerable amount of bile duct proliferation and nuclear abnormalities.

Discussion — The finding that diets containing 53% casein–gelatin mix and 8 or 16% herring oil

TABLE 1. Composition of test diets (%).

Ingredient	Diet								
	1	2	3	4	5	6	7	8	9
Protein mix[a]	35.56	44.44	53.33	35.56	44.44	53.33	35.56	44.44	53.33
Herring oil	8.00	8.00	8.00	16.00	16.00	16.00	24.00	24.00	24.00
Starch	26.67	17.80	8.90	26.67	17.80	8.90	26.67	17.80	8.90
Cellulose	22.45	22.44	22.45	14.45	14.44	14.45	6.45	6.44	6.45
Mineral mix[b]	4.00								
Vitamin mix[c]	2.00								
Methionine (L)	0.20								
Tryptophan (L)	0.10								
Choline chloride (70%)	1.00								
Vitamin E conc.[d]	0.20								
Kcal/100 g[e]	296	296	296	368	368	368	440	440	440
% Protein calories	43.3	54.1	64.8	34.8	43.5	52.2	29.2	36.4	43.6

[a]70% casein, 30% gelatin, Nutritional Biochemicals. Actual protein levels were approximately 90% protein mix levels.

[b]Bernhart–Tomarelli salt mix.

[c]Supplies in mg/kg diet: thiamine, 64; riboflavin, 144; niacinamide, 512; biotin, 1.6; co-pantothenate, 288; pyridoxine, 48; folic acid, 19.2; menadione, 16; cobalamine, 0.16; L-inositol, 2500; ascorbic acid, 1200; PABA, 400; vitamin A and D were added at 25 and 4 IU/g, respectively.

[d]Supplies 660 IU vitamin E/kg.

[e]Calculated with starch and protein = 4 kcal and lipid = 9 kcal/g.

TABLE 2. The influence of test diets on weight gain, liver levels of glycogen and lipid, liver histology, and liver size.

Diet	Avg. final wt(*g*)[1]	Liver glycogen (%)[1]	Liver glycogen Histological score[2]	Nuclear abnormalities	Bile-duct proliferation[2]	Parenchymal cell injury[3]	Liver lipid(%)[1]	liver size[1] (*g/100-g fish*)
1	40.6±1.0[a]	3.1±0.2[c]	1.87	0–1	26	0	2.3±0.2[c,d]	1.4±0.1[a]
2	47.8±4.0[b]	5.0±1.4[d,e]	2.13	0–1	20	0	1.7±0.3[a,b]	1.7±0.2[b]
3	51.1±2.5[b,c,d]	6.2±0.8[e]	2.60	2	94	+	1.5±0.1[a]	1.9±0.2[b]
4	42.0±1.4[a]	1.6±0.4[a]	1.53	0	26	0	1.9±0.2[a,b,c]	1.2±0.1[a]
5	51.8±1.8[b,c]	1.8±0.6[a,b]	1.53	0–1	26	0	2.3±0.4[c,d]	1.3±0.1[a]
6	54.3±1.8[c,d]	3.0±0.8[b,c]	2.33	1½	40	+	1.6±0.5[a,b]	1.7±0.2[b]
7	53.6±2.8[c,d]	1.8±0.6[a,b]	1.53	0–1	26	0	2.4±0.1[d]	1.4±0.1[a]
8	52.9±1.2[c,d]	1.5±0.3[a]	1.66	0–1	20	0	1.8±0.1[a,b,c]	1.3±0.1[a]
9	51.2±3.4[b,c,d]	3.8±0.7[c,d]	2.06	0	7	0	2.0±0.4[b,c,d]	1.7±0.3[b]

[1]Values given are averages of three lots of fish ± standard deviation. Values with same superscript are not significantly different ($P < .05$)

[2]Average histological scores of 15 livers/diet: liver glycogen rated 1 (low), 2, 3; nuclear abnormalities graded 0 (normal), 2, 3; bile-duct proliferation, 0 (normal), 1, 2, 3, 4, 5. (Bile-duct proliferation scores shown are the total of each diet divided by 15 × 100.)

[3]Lots in which appreciable parenchymal cell injury was found are indicated (+).

(3/1). The solvent was removed by vacuum evaporation and the residue dried at 70 C to constant weight. The produced abnormal livers with high glycogen levels was unexpected. The casein–gelatin diet, with the methionine and tryptophan supplements, supplied recognized essential amino acids (Halver et al. 1957; Halver and Shanks 1960; DeLong et al. 1962). If an amino acid imbalance was responsible for the abnormal liver conditions, increasing the lipid level of the diet was apparently able to counteract the effects. Diet 9, which contained 53% protein mix and 24% lipid, produced normal livers. The high fat level would reduce the need to utilize protein for energy, but it is not clear how this would prevent the effects of a gross amino acid imbalance.

High carbohydrate diets have been associated with increased liver glycogen levels and slow growth in trout (Phillips 1969), but the authors are not aware of high protein diets producing this condition. Diet 3 supplied 65–70% of its total calories as protein and diet 6, 50–55%. In the trial reported here, fish receiving 27% dietary starch (Diets 1, 4, and 7) had lower levels of liver glycogen than trout fed 9% starch (Diets 3, 6, and 9). The high percentage of poorly digested starch calories in Diets 1 and 4 (32 and 26%) probably caused the slow gain in these groups of trout by decreasing total amount of available energy. Growth was not seriously inhibited by the factor(s) causing the liver injury, as both Diets 3 and 6 had very good growth rates. Fish with the poorest growth (Diets 1 and 4) had "normal" livers.

The reason(s) for the high levels of liver glycogen and abnormal liver histology of the lots fed 53% protein mix is unknown. If an imbalance of nutrients was responsible, it is not apparent from our present knowledge of fish nutrition. The possibility of a toxic contaminant in the protein source exists, but this explanation is confused by the lack of toxicity symptoms seen in fish fed Diet 9. The significance of these liver abnormalities is difficult to assess. In this short-term feeding trial no deleterious effect on growth was observed. In a long-term evaluation, however, reproduction, stamina, and longevity could be impaired. Regardless of the cause(s) of the toxicity noted, the observed liver abnormalities demonstrate the value of measuring more than growth in feeding trials.

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CARROL, N. V., R. W. LONGLEY, AND J. H. ROE. 1956. The determination of glycogen in liver and muscle by use of anthrone reagent. J. Biol. Chem. 220: 583–587.

CASTELL, J. D., R. O. SINNHUBER, J. H. WALES, AND D. J. LEE. 1972. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): growth, feed conversion and some gross deficiency symptoms. J. Nutr. 102: 77–85.

DELONG, D. C., J. E. HALVER, AND E. T. MERTZ. 1958. Nutrition of salmonoid fishes VI. Protein requirements of chinook salmon at two water temperatures. J. Nutr. 65: 589–599.

1962. Nutrition of salmonoid fishes X. Quantitative threonine requirements of chinook salmon at two water temperatures. J. Nutr. 76: 174–178.

HALVER, J. E. 1957. Nutrition of salmonoid fishes III. Water-soluble vitamin requirements of chinook salmon. J. Nutr. 62: 225–243.

HALVER, J. E., D. C. DELONG, AND E. T. MERTZ. 1957. Nutrition of salmonoid fishes V. Classification of essential amino acids for chinook salmon. J. Nutr. 63: 95–105.

HALVER, J. E., AND W. E. SHANKS. 1960. Nutrition of salmonoid fishes VIII. Indispensable amino acids for sockeye salmon. J. Nutr. 72: 340–346.

LEE, D. J., J. N. ROEHM, T. C. YU, AND R. O. SINNHUBER. 1967. Effect of ω3 fatty acids on the growth rate of rainbow trout, *Salmo gairdnerii*. J. Nutr. 92: 93–98.

PHILLIPS, A. M., JR. 1969. Nutrition, digestion and energy utilization, p. 391–432. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Academic Press, New York, N.Y.

An Improved Method of Using One-way Glass to Assess Visual Discrimination in Fish

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SOSIAK, A. J. 1973. An improved method of using one-way glass to assess visual discrimination in fish. J. Fish. Res. Board Can. 30: 1021–1022.

A method using one-way glass for presenting a test fish with a choice of two fish as visual stimuli was developed during a study to test visual discrimination by sunfish. This method is more effective than a previous one-way glass system in that it prevents habituation by test fish to their reflections in the one-way glass and makes nonvisual recognition cues less likely.

SOSIAK, A. J. 1973. An improved method of using one-way glass to assess visual discrimination in fish. J. Fish. Res. Board Can. 30: 1021–1022.

Lors d'une étude sur le discernement chez le crapet, nous avons mis au point une méthode utilisant un verre à sens unique pour offrir à un poisson expérimental un choix de deux poissons comme stimulus visuel. Cette méthode est plus efficace que le système d'un verre à sens unique utilisé précédemment, parce qu'elle empêche les poissons expérimentaux de s'accoutumer à leur réflection sur le verre, et rend moins probable la présence de stimuli non visuels.

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VISUAL discrimination in a test fish can be demonstrated by simultaneously presenting two fish as visual stimuli and recording responses to the two. A nonrandom response pattern by the test fish suggests an ability to visually discriminate between the two stimuli. If one-way glass prevents the stimulus fish from seeing the test fish, then the possibility of stimulus fish reaction to the test fish influencing the behavior of the latter is eliminated.

Steele and Keenleyside (1971) developed a system for testing visual discrimination in fish by using one-way glass. Two inadequacies in their design reduce its effectiveness. First, as the test fish is placed in the apparatus for 1 day prior to testing, and during this time can see its reflection in the one-way glass, habituation to its own reflection may occur. Second, as the authors noted, conspecific recognition cues, apparently auditory, may occur in the absence of visual stimuli. Their design therefore, because test and stimulus fish are all in the same tank, allows nonvisual as well as visual cues.

The method herein described eliminates habituation, reduces the likelihood of nonvisual recognition cues, and uses a slightly different method of stimulus introduction.

Apparatus and testing procedure — Each test fish is placed alone in a 45-liter glass tank (54 × 26 × 31 cm deep). Approximately 4 cm of gravel covers the bottom of the tank, and air is supplied by a yellow air stone located centrally on a 54-cm side. Buff colored paper is taped to three sides of the tank with some overlap onto the fourth side (Fig. 1), to reduce reflections within the tank. A rectangular opening (7.5 × 19.5 cm) is made in the paper at both ends of the tank. Vertical wax pencil lines on the 54-cm sides divide the tank lengthwise into three equal sections.

The two stimulus fish are placed separately in two and 6.5-liter tanks (15.5 × 15.5 × 27 cm deep, tanks A B, Fig. 1), 21 cm from either end of the 45-liter tank.

Sheets of one-way glass (30 × 30 cm) are placed at a 45° angle between the stimulus tanks and the testing tank (Fig. 1). A second pair of tanks (C and D) are placed at right angles to a line connecting the centers of the stimulus and testing tanks. Tanks A, B, C, and D are as nearly identical as possible in all respects. They are of equal dimensions, each contains about 2 cm of gravel, and each is aerated with a yellow air stone. Black cloth covers three sides of each tank, and the open side (through which the interior of the tank is visible) is turned towards the one-way glass. Tanks A and B receive illumination from two 150-w photoflood lights, and tanks C and D from two 15-w desk lamps. At a given time only one pair of lamps is illuminated, and it is possible to switch illumination from one pair to the other by turning a three-way toggle switch. Cardboard and aluminum foil reflectors around each of the small tanks prevent light from the illuminated pair of tanks reaching the pair in darkness. Thus, the only light to leave the small tanks is directed towards the one-way glass. A cardboard box covered with black cloth is placed over the testing tank (Fig. 1). This prevents light from reaching the testing tank except through two rectangular openings at either end aligned with openings in the paper covering the testing tank. A 5-inch observation slit is cut in one side of the box.

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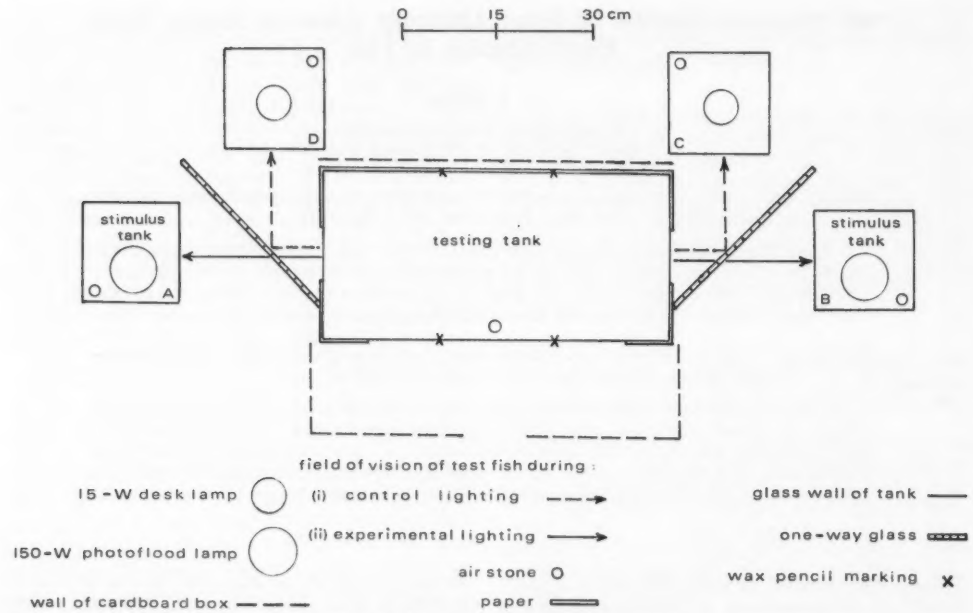

FIG. 1. The experimental apparatus.

Two lighting conditions were used. Under control lighting, only tanks C and D were illuminated, and the reflections of these tanks off the one-way glass were visible to the test fish in the testing tank. The stimulus tanks (A and B) were in darkness and were therefore not visible. Under experimental lighting, only the stimulus tanks were illuminated and were thus visible to the test fish through the one-way glass.

In each test the test fish was first observed under control lighting to reveal any preference for either end of the tank. Two observation periods with experimental lighting followed, the second with stimulus fish reversed in position. More details on testing procedure are given by Sosiak (1972).

Results — Preliminary results using this method (Sosiak 1972) indicate its effectiveness. Male and female bluegills (*Lepomis macrochirus*) visually discriminated conspecific females from female pumpkinseeds, *Lepomis gibbosus* ($n = 6$, $P < 0.05$), in one series of observations. Female pumpkinseeds discriminated conspecific females from female black crappie, *Pomoxis nigromaculatus* ($n = 8$, $P < 0.05$). However, male pumpkinseeds did not discriminate conspecifics from either bluegills or black crappie.

Discussion — Rotation of the one-way glass through 45° serves two functions. First, it prevents stimulus fish from seeing and responding to their reflections; their responses may otherwise influence test fish behavior. Second, for the test fish to see tanks C and D under control lighting requires that the one-way glass be rotated. Control lighting allows test fish and stimulus fish to become accustomed to the apparatus without the former seeing their reflections as in previous designs. Switching from control lighting to experimental lighting, or vice versa, allows a visual stimulus to be introduced or withdrawn as desired.

Although this technique was developed for testing visual recognition of conspecifics by fish, it may be adapted to visual discrimination work with non-aquatic organisms.

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SOSIAK, A. J. 1972. Colour patterns of *Lepomis gibbosus* and *L. macrochirus* as species recognition cues. B.Sc. Thesis. Brock Univ., St. Catharines, Ont. 55 p.

STEELE, R. G., AND M. H. A. KEENLEYSIDE. 1971. Mate selection in two species of sunfish (*Lepomis gibbosus* and *L. megalotis*). Can. J. Zool. 49: 1541–1548.

The *p*H Activity Relations of Two LDH Homotetramers From Trout Liver and Their Physiological Significance

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TSUYUKI, H., AND S. N. WILLISCROFT. 1973. The *p*H activity relations of two LDH homotetramers from trout liver and their physiological significance. J. Fish Res. Board Can. 30: 1023–1026.

The LDH homotetramers from the liver tissues of B″B″ and B′B′ steelhead trout phenotypes were each demonstrated to possess two *p*H optima, the usually described broad one spreading over 1.0 units in the alkaline region, and a new extremely narrow one spread over 0.128 units in the physiological range. The narrow *p*H optimum of LDH-B″$_4$ in the lactate to pyruvate reaction exhibited an optimum at 7.302 and that of LDH–B$_4$ at 7.430. In the opposite reaction, LDH-B″$_4$ and -B$_4$ passed through equally sharp minima at *p*H 7.331 and 7.428, respectively. At their respective narrow *p*H optima, LDH-B″$_4$ exhibited a lactate conversion rate 3.6 times higher than that of LDH-B$_4$.

The possible physiological significance of the narrow *p*H optima to poikilotherms and the differential lactate conversion rate to relative swimming endurance between the two phenotypes are discussed.

TSUYUKI, H., AND S. N. WILLISCROFT. 1973. The *p*H activity relations of two LDH homotetramers from trout liver and their physiological significance. J. Fish. Res. Board Can. 30: 1023–1026.

Les homotétramères de LDH provenant du tissu hépatique des phénotypes B″B″ et B′B′ de la truite steelhead possèdent chacun deux optimums de *p*H, l'optimum étendu, déjà connu et communément décrit, qui couvre 1.0 unité dans la région alcaline, et un nouveau, extrêmement étroit, qui couvre 0.128 unité sur la gamme physiologique. Dans la réaction lactate à pyruvate, la LDH-B″$_4$ montre un optimum étroit de *p*H à 7.302, et la LDH-B$_4$ à 74.30. Dans la réaction inverse, LDH-B″$_4$ et -B$_4$ passent par un minimum tout aussi brusque à des *p*H respectifs de 7.331 et 7.428. A leur optimum étroit de *p*H respectif, LDH-B″$_4$ convertit le lactate à un taux 3.6 fois plus élevé que LDH-B$_4$.

Les auteurs examinent, chez les deux phénotypes, la signification physiologique possible de l'optimum étroit de *p*H pour les poikilothermes, et l'effet que peut avoir le taux différentiel de conversion du lactate sur l'endurance à la nage.

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THE historical advances in lactate dehydrogenase (LDH) research, the eventual recognition of the existence of multiple molecular forms, the molecular basis of subunit structure, and several possible physiological implications of the isozymes are described in the reviews of Markert (1968) and Markert and Whitt (1968). Other aspects of investigations are covered in recent articles by Wuntch and Goldberg (1970) and in a host of other publications. Some of the kinetic properties of tissue specific LDH have been described by Fondy et al. (1964), Anderson et al. (1964), Kaplan (1965), Battellino et al. (1968) and Zinkham et al. (1964).

The finding of two allelic LDH subunits in the liver of rainbow trout (Williscroft and Tsuyuki 1970) provided a source of distinct homotetramers from the same tissue type for a reinvestigation o *p*H-activity relationships and some other kinetic properties. The possible implication of these properties on current response (Northcote 1969) and temperature adaptation of poikilotherms is discussed.

The designations C and C′ used previously (Williscroft and Tsuyuki 1970) for the rainbow trout phenotypes have been revised to B″ and B′, respectively, on the basis of further study (G. S. Bailey and H. Tsuyuki unpublished data). The homotetrameric LDHs from trout liver are designated B″$_4$ and B′$_4$.

Materials and methods — Fresh and frozen liver tissues were acquired from the B″B″ and B′B′ phenotypes of rainbow trout (*Salmo gairdneri*) as previously described (Williscroft and Tsuyuki 1970) and from the anadromous

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steelhead trout (*S. gairdneri*) from the Thompson River in British Columbia. The tissue was homogenized in nine volumes of 0.05 M sodium phosphate buffer, *p*H 7.5, and centrifuged for 20 min at 27,000 × g. The supernatant was diluted a further ninefold with the same buffer.

All *p*H measurements were carried out with a Beckman Research *p*H meter with a relative accuracy of 0.001 *p*H units and a reproducibility of 0.0005 units. Calibration curves of temperature versus *p*H for 0.05 M phosphate buffer were prepared in triplicate and averaged over a temperature range of 0–25 C. The *p*H of the reaction mixture was checked routinely before and after LDH activity measurements.

LDH activity was measured by a slight modification of the method of Zewe and Fromm (1962). For temperature-controlled activity measurements, a Beckman DB Recording Spectrophotometer equipped with a Haake thermostated circulating water bath, and a 1-cm light path cuvette was used.

Results and discussion — In the lactate to pyruvate direction at 24.8 C, LDH-B″$_4$ displays two *p*H optima, a broad maximum spreading over more than one *p*H unit beyond *p*H 7.75, and an extremely narrow maximum at *p*H 7.302 (Fig. 1). Similarly, LDH-B′$_4$ displayed a broad *p*H optimum in addition to a narrow one at *p*H 7.430. Preliminary experiments showed that the *p*H optima remained constant over a range of 3–25 C. It will be noted that *p*H measurements must be made beyond an accuracy of 0.01 units. At their respective *p*H optima, the in vitro rate of conversion of lactate, at substrate saturation and at a coenzyme level almost 7 and 4 fold more than those found, respectively, in equivalent amounts of rat liver (Lowry et al. 1961) and fish liver (Shimizu et al. 1969) tissues, was approximately 3.6 times greater with LDH-B′$_4$ than with -B′$_4$. In the reverse direction, pyruvate to lactate, both LDH-B″$_4$ and -B′$_4$ possess sharp minima at *p*H 7.331 and 7.428, respectively (Fig. 2).

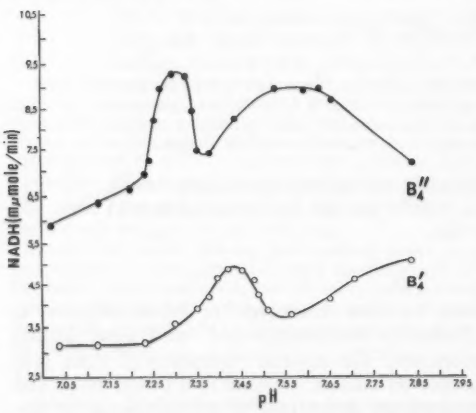

FIG. 1. The effect of *p*H on activity of trout liver LDH in the lactate to (pyruvate) direction. B″$_4$ refers to the liver LDH homotetramer from the B″B″ trout phenotype and B′$_4$ to that from the B′B′ phenotype. The reaction mixtures consisted of 0.08 ml of B″$_4$ enzyme or 0.15 ml of B′$_4$ enzyme, 0.1 ml of a 1.6011 mM solution of NAD, 0.1 ml of neutralized 0.27M, hydroxylamine hydrochloride, and 0.1 ml of a 0.432M solution of lithium lactate in a final volume of 2.70 ml with 0.05M, sodium phosphate buffer. The lithium lactate solution was brought to the appropriate *p*H and added last. The temperature of the reaction was held at 24.8 C. The change in absorption at 340 mμ during the initial phase of the reaction was used to calculate the reaction rate.

It is emphasized that the activity in both phenotypes passes through a maximum within a very narrow *p*H range of about 0.1–0.15 units (Fig. 1). This is in the same order of magnitude as the shift 0.1–0.14 *p*H unit (Reeves and Wilson 1969) caused by a change in temperature of 10 C, the actual seasonal difference in water temperature observed at Kokanee Creek (Northcote 1969). The recognition that the LDH activity passes through a maximum within such a narrow *p*H range suggests the existence in poikilotherms of a basic mechanism by which biochemical reaction rates caused by temperature variations within the physiological range can be compensated.

It is also pointed out that in both phenotypes, the *p*H dependency of liver LDH activity is greatest around the physiological *p*H of rainbow trout. The magnitude of change in blood *p*H (Black et al. 1959) by the lactic acid formed during strenuous muscular exercise is also approximately in the range of the narrow *p*H optima observed in these experiments. Thus, increasing acidity through lactic acid production could well be countered by the increasing rate of conversion of lactic acid at least till the *p*H of maximum liver LDH activity is attained. The recent report of Bilinski and Jonas (1972) that fresh liver slices of rainbow trout decarboxylates $^{14}CO_2$H-lactate at a rate for exceeding that of white and red muscle and kidney tissue, tends to support the major role played by the liver tissue in the conversion of lactate to pyruvate despite unfavorable literature reports (Black et al. 1966; Dando 1969).

The changes in *p*H caused either by variations in temperatures within the physiological range or by lactic acid production during strenuous muscular activity are small and can be effective in the control of LDH catalyzed reaction rate only if a narrow *p*H optimum exists. The second *p*H optimum is not only well above the physiological *p*H but also so broad in range that small *p*H changes will have no noticeable effect on reaction rate.

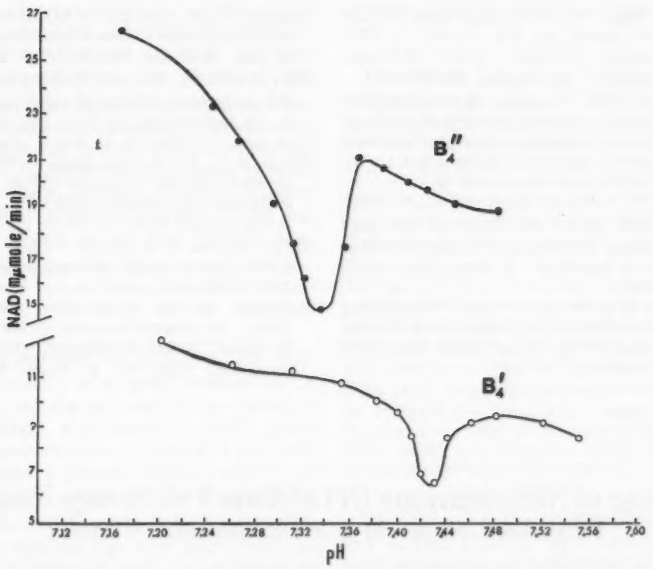

FIG. 2. The effect of *p*H on activity of trout liver LDH in the pyruvate to lactate direction. The reaction mixtures consisted of .08 ml of each of the B''_4 and B'_4 enzymes, 0.1 ml of a 1.6011 mM solution of NADH, and 0.1 ml of a 39.7 mM solution of sodium pyruvate in a final volume of 2.7 ml with 0.05M sodium phosphate buffer. The substrate was added last. The temperature of the reaction mixture was maintained at 10.1 C.

In vitro, liver LDH-B''_4 at saturating lactate concentrations has both a lower *p*H optimum and a better than threefold higher rate of lactate turnover than LDH-B'_4. Rainbow trout with the LDH-B''_4 phenotype appears indigenous to environmental conditions characterized chiefly by rapid water flow (Northcote et al. 1970). Samplings of populations of rainbow trout which displayed a tendency to swim against the current (Northcote 1969) also proved later to be associated with a high proportion of LDH-B''_4 phenotypes (Northcote et al. 1970). The higher lactate conversion rate of LDH-B''_4 could well confer to this phenotype a superior swimming endurance. Studies in this direction are in progress.

ANDERSON, S. R., J. R. FLORINI, AND C. S. VESTLING. 1964. Rat liver lactate dehydrogenase. J. Biol. Chem. 239: 2991–2997.

BATTELLINO, L. J., F. R. JAIME, AND A. BLANCO. 1968. Kinetic properties of rabbit testicular lactate dehydrogenase isozyme. J. Biol. Chem. 243: 5185–5192.

BILINSKI, E., AND R. E. JONAS. 1972. Oxidation of lactate to carbon dioxide by rainbow trout (*Salmo gairdneri*) tissues. J. Fish. Res. Board Can. 29: 1467–1471.

BLACK, E. C., W. G. CHIU, F. D. FORBES, AND A. HANSLIP 1959. Changes in *p*H, carbonate and lactate of the blood of yearling Kamloops trout, *Salmo gairdneri*, during and following severe muscular exercise. J. Fish. Res. Board Can. 16: 391–402

BLACK, E. C., G. T. MANNING, AND K. HAYASHI. 1966. Changes in levels of hemoglobin, oxygen, carbon dioxide, pyruvate, and lactate in venous blood of rainbow trout (*Salmo gairdneri*) during and following severe muscular activity. J. Fish. Res. Board Can. 23: 783–795.

DANDO, P. R. 1969. Lactate metobolism in fish. J. Mar. Biol. Ass. U.K. 49: 209–223.

FONDY, T. P., A. PESCE, I. FREEDBERG, F. STOLZENBACH, AND N. O. KAPLAN. 1964. The comparative enzymology of lactate dehydrogenases. II. Properties of the crystalline HM_3 hybrid from chicken muscle and of H_2M_2 hybrid and H_4 enzyme from chicken liver. Biochemistry 3: 522–530.

KAPLAN, N. O. 1965. Evolution of dehydrogenases, p. 243–277. *In* V. Bryson and N. J. Vogel [ed.] Evolving genes and proteins. Academic Press, Inc., New York, N.Y.

LOWRY, O. H., J. U. PASSONNEAU, D. W. SCHULZ, AND M. K. ROCK. 1961. The measurement of pyridine nucleotides by enzymatic cycling. J. Biol. Chem. 236: 2746–2755.

MARKERT, C. L. 1968. The molecular basis for isozymes. Ann. N.Y. Acad. Sci. 151: 14–40.

MARKERT, C. L., AND G. S. WHITT. 1968. Molecular varieties of isozymes. Experientia 24: 977–991.

NORTHCOTE, T. G. 1969. Patterns and mechanisms in the lakeward migratory behaviour of juvenile trout, P. 181–204. *In* T. G. Northcote [ed.] Salmon and trout in streams. H. R. MacMillan lectures in fisheries, Univ. of British Columbia, Vancouver, B. C.

NORTHCOTE, T. G., S. N. WILLISCROFT, AND H. TSUYUKI. 1970. Meristic and lactate dehydrogenase genotype differences in stream populations of rainbow trout below and above a waterfall. J. Fish. Res. Board Can. 27: 1987–1995.

REEVES, R. B., AND T. L. WILSON. 1969. Intracellular *p*H in bullfrog striated and cardiac muscle as a function of body temperature. Fed. Proc. Amer. Soc. Exp. Biol. 28: 782.

SHIMIZU, C., K. ABE, AND F. MATSUURA. 1969. Levels of oxidized and reduced NAD and NADP in tissues of fish. Bull. Jap. Soc. Sci. Fish. 35: 1034–1040.

WILLISCROFT, S. N., AND H. TSUYUKI. 1970. Lactate dehydrogenase systems of rainbow trout — evidence for polymorphism in liver and additional subunits in gills. J. Fish. Res. Board Can. 27: 1563–1567.

WUNTCH, T., AND E. GOLDBERG. 1970. A comparative physico-chemical characterization of lactate dehydrogenase: isozymes in brook trout, lake trout and their hybrid splake trout. J. Exp. Zool. 174: 233–252.

ZEWE, V., AND H. J. FROMM. 1962. Kinetic studies of rabbit muscle lactate dehydrogenase. J. Biol. Chem. 237: 1668–1675.

ZINKHAM, W. H., A. BLANCO, AND L. J. CLOWRY. 1964. An unusual isozyme of lactate dehydrogenase in mature testes: localization, ontogeny, and kinetic properties. Ann. N. Y. Acad. Sci. 121: 571–588.

Discharge of Nitrilotriacetate (NTA) from Two Sewage Treatment Facilities Located in a Midcontinental Climate

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RUDD, J. W. M., B. E. TOWNSEND, AND R. D. HAMILTON. 1973. Discharge of nitrilotriacetate (NTA) from two sewage treatment facilities located in a midcontinental climate. J. Fish. Res. Board Can. 30: 1026–1030.

Concentrations of NTA entering the Red and Assiniboine rivers from two Winnipeg sewage treatment facilities were monitored. The activated sludge plant usually discharged <0.5-mg NTA/liter throughout the year. The aerated sewage lagoon discharged <20-μg NTA/liter during summer. It was believed that the NTA passed through the lagoon essentially unchanged during winter (1800-μg NTA/liter) when degradation rates must have been extremely low. No NTA was detected in river water downstream from either of these facilities. It was concluded that even under extreme environmental conditions NTA discharge did not present a hazard to either local aquifers or the local biota.

RUDD, J. W. M., B. E. TOWNSEND, AND R. D. HAMILTON. 1973. Discharge of nitrilotriacetate (NTA) from two sewage treatment facilities located in a midcontinental climate. J. Fish. Res. Board Can. 30: 1026–1030.

Les concentrations de NTA déversé dans les rivières Rouge et Assiniboine par deux installations de traitement d'eaux d'égout de Winnipeg ont été sous observation continue. L'usine de boues activées déverse ordinairement <0.5 mg de NTA/litre à l'année longue. La lagune d'aération déverse <20 μg de NTA/litre en été. Nous croyons qu'en hiver le NTA demeure pratiquement inchangé (1800 μg de NTA/litre) après son passage dans la lagune, et qu'il a atteint le niveau de 1800 μg de NTA/litre au cours de l'hiver où les taux de dégradation ont dû être extrêmement bas. Nous n'avons pas décelé de NTA dans l'eau de rivière en aval de ces installations. Nous en concluons que, même dans des conditions environnementales extrêmes, le déversement de NTA ne présente pas de danger, ni pour les aquifères ni pour les biota locaux.

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TRISODIUM nitrilotriacetate (NTA) is currently being used as a replacement for phosphate in some detergents marketed in Canada. Concern has been expressed regarding the possible effects of this strong chelator and its degradation products upon man and the environment (Epstein 1972a, b). The most obvious route for this chemical to enter the environment and, possibly, drinking water supplies is via sewage systems. Sewage treatment lagoons are common in central Canada, in spite of the fact that they must operate at the low ambient temperatures typical of the "midcontinental" temperature regime. Such temperatures are far from the optima associated with most sewage treatment processes.

Previously we reported in detail on the construction and operation of a model sewage lagoon (Rudd 1972) and on the reaction of the model to NTA loading (Rudd and Hamilton 1972). From these studies experimentation involving a full scale lagoon was considered impractical for a number of reasons. The increased usage of NTA in Canadian detergents has eliminated many of these objections and indeed has supplied the very experiment we would have wished from the start. In this report we present results of NTA determinations obtained during a survey of two sewage treatment facilities and the associated receiving rivers of the Winnipeg area.

The aerated cells of the Charleswood Lagoon are operated by the City of Winnipeg as experimental facilities designed to test the efficacy of the various aeration techniques under severe winter conditions (Anon. 1969). Throughout the fall, winter, and summer of 1971–72 samples were obtained from this lagoon system, from an activated sludge plant that serves 90% of the city, and from the rivers into which the two systems discharge. Sampling locations are presented in Fig. 1. The sampling period was chosen to cover the seasons of the year considered to provide the least optimal operating conditions for the lagoon. Samples were transported to the laboratory at temperatures that permitted neither freezing of the samples nor significant biological activity, and analyzed as described previously (Rudd and Hamilton 1972). Samples of incoming raw sewage could not be analyzed for their NTA content because of interferences from other compounds (Murray and Povoledo 1971; Rudd and Hamilton 1972). The basic results of this survey are presented in Table 1.

In Table 2 data are presented confirming that the aerated cells of the Charleswood Lagoon, in spite of their experimental character, were operating normally with respect to the standard assays of efficiency. It should be noted, however, that in late

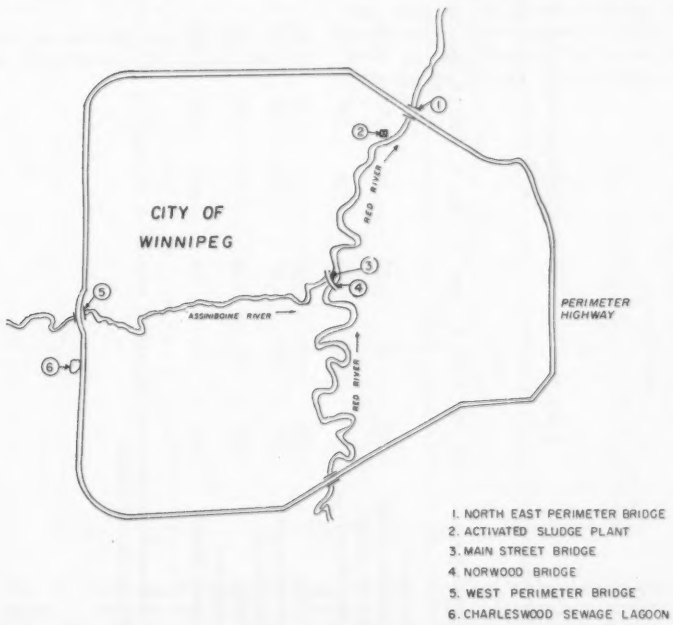

FIG. 1. NTA river and sewage treatment sampling sites in the City of Winnipeg.

TABLE 1. Concentration of NTA(μg/liter) in two Winnipeg sewage treatment plants and receiving rivers.

Sampling sites	1971			1972										
	Oct. 10	Nov. 4	Dec. 21	Jan. 7	Feb. 11	Feb. 26	Mar. 16	Apr. 14	May 5	May 26	June 16	July 7	July 28	Aug. 18
North east perimeter bridge	<20[a]	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Activated sludge plant effluent	175	250	180	305	255	140	380	215	570	355	435	395	615	830
Main St. bridge	<20	<20	<20	<20	<20	<20	–	<20	<20	<20	–	–	–	<20
Norwood bridge	<20	<20	<20	<20	<20	<20	–	<20	<20	<20	–	<20	–	<20
West perimeter bridge	<20	<20	<20	<20	<20	<20	–	<20	<20	<20	–	<20	<20	<20
Charleswood lagoon effluent	550	–[b]	765	1650	1510	1800	1775	1000	980	<20	<20	<20	–	–

[a]Concentrations shown as <20 μg/liter were below the sensitivity of the analytical method.
[b](–) denotes no sample.

1971 certain modifications were instituted that resulted in a much increased retention time of sewage in the aerated cells of the lagoon, thus changing its characteristics from those the model was designed to mimic (Rudd and Hamilton 1972).

In Fig. 2 the mean monthly ambient air temperature and, where available, the actual lagoon temperature are contrasted with the NTA content of the lagoon effluent. Discharge of NTA was very low at high ambient temperatures and very high at temperatures below 10 C. This was not a result of a lack of dissolved oxygen during winter (Table 2). Without access to influent NTA concentrations, one cannot state precise percentages of degradation during the period when the lagoon operated below 10 C but, in comparison with results obtained from the model, one would suspect that the NTA may have been passing through the lagoon essentially unchanged.

TABLE 2. BOD_5 and dissolved oxygen concentrations in the effluent of the aerated cells of the Charleswood Sewage Lagoon during the period of NTA sampling.

	Influent BOD_5 (*mg/liter*)	Effluent BOD_5 (*mg/liter*)	Dissolved oxygen (*mg/liter*)
Dec. 21/71	250[a]	16	12.0
Jan. 7/72	242	26	9.5
Feb. 9/72	203	50	6.3
Feb. 24/72	240	18	4.8
Mar. 16/72	228	33	4.4
Apr. 13/72	108	14	8.4
May 4/72	180	17	10.2
May 25/72	276	19	7.7
June 16/72	144	82	–

[a]The data appearing in this table were supplied by the City of Winnipeg Waterworks and Waste Disposal Division.

The worst possible environmental consequences would occur during periods of high NTA discharge into low levels of receiving waters. In the Winnipeg area these conditions coincide during midwinter. Due to decreased runoff during winter, the flow rate of the lagoon dropped and the influent sewage was more concentrated (BOD_5 of the influent rose by a factor of about 2.5). However, the observed NTA concentrations rose by two orders of magnitude resulting in maximal NTA discharge. Winter is also known to be the lowest period of river flow (Anon. 1971, 1972). In spite of this conjunction of adverse conditions, we have been unable to detect the presence of NTA in the rivers of the area and we can only conclude that either NTA was being degraded in the receiving waters or it was being diluted below the sensitivity of the assay method (<20 μg/liter). Preliminary calculations of river flow and NTA discharge have indicated that the latter case appears to be more likely. In either event, the terminal concentration of NTA under present levels of usage appears to be so low as to be of little danger to the aquatic biota (Christie 1970;

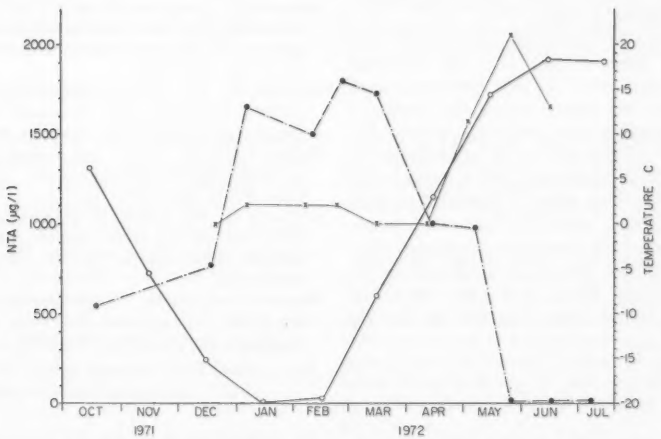

FIG. 2. Response of effluent NTA concentration from the Charleswood sewage lagoon to seasonal fluctuations in temperature; NTA concentration, broken line; effluent temperature, crosses; and mean monthly ambient temperature, open circles.

Flannagan 1971; Jancović and Mann 1969; Sprague 1968), and would even appear to be of little danger to those individuals so insensitive to other human health problems as to drink untreated river water directly.

Most of the drinking water for the Winnipeg area originates from a source that is not exposed to sewage pollution. However, some water (most of which is used for air conditioning systems) is pumped from local aquifers and, as a result of this, a drawdown cone has developed in central Winnipeg. This results in a net flow of river water into the aquifers. This flow is most pronounced during the summer months and during high water periods in the rivers (Render 1970). However, in view of the low concentrations of NTA in river water and the usage of this water, this ground water flow would not appear to be an important source of toxicants such as NTA. River water also enters an aquifer adjacent to the Red River channel in North Winnipeg during spring flood periods. This also appears to be of little importance since this water is flushed back into the river when the aquifer head builds up or when the river returns to normal levels (F. W. Render personal communication). At flood stage the lagoon and the activated sludge plant are also subject to increased flow and therefore maximal dilution of incoming NTA would occur. This in-plant dilution of NTA would then be followed by a huge dilution as the discharged NTA is delivered to receiving waters. Therefore, any net flow to the aquifers as a result of high water would contain very small amounts of NTA that, in the case of North Winnipeg, would be lost when the aquifer began to discharge once more.

It may therefore be concluded that: firstly, the activated sludge plant of the City of Winnipeg discharged less than 1-mg NTA/liter maximally and usually discharged less than one-half this amount; secondly, the lagoon sewage treatment system discharged very low NTA levels (<20 µg/liter) during warm weather but discharged NTA apparently unchanged (1800-µg/liter) when it operated at temperatures below 10 C (7 months of the year, Anon. 1969); thirdly, even under extreme conditions NTA was not found to be detectable (<20-µg NTA/liter) in the receiving waters and thus does not present a hazard to the local aquifers or to the local biota.

Acknowledgments — We thank Mr M. Pitze of the Freshwater Institute for the analyses of NTA samples. Gratitude is also extended to the City of Winnipeg Waterworks and Waste Disposal Division and, in particular, Mr B. Duchominsky for providing us with necessary data and for allowing us access to their sewage treatment facilities. Mr F. W. Render of the Department of Mines and Natural Resources, Province of Manitoba, kindly provided us with valuable information concerning the ground water flow systems in the Winnipeg area.

ANON. 1969. Evaluation of aerated lagoons in Metropolitan Winnipeg. Metropolitan Corporation of Greater Winnipeg, Waterworks and Waste Disposal Division, Winnipeg, Man. 71 p.

1971. Water survey of Canada. Inland Waters Branch, Dep. Environ., Ottawa, Can. 157 p.

1972. Water survey of Canada. Inland Waters Branch, Dep. Environ., Ottawa, Can. (In Press)

CHRISTIE, A. E. 1970. Trisodium nitrilotriacetate and algae. Water Sewage Works 117: 58–59.

EPSTEIN, S. S. 1972a. Toxicological and environmental implications on the use of nitrilotriacetic acid as a detergent builder – I. Int. J. Environ. Stud. 2: 291–300.

1972b. Toxicological and environmental implications on the use of nitrilotriacetic acid as a detergent builder – II. Int. J. Environ. Stud. 3: 13–21.

FLANNAGAN, J. F. 1971. Toxicity evaluation of trisodium nitrilotriacetate to selected aquatic invertebrates and amphibians. Fish. Res. Board Can. Tech. Rep. 258: 21 p.

JANCOVIĆ, M., AND H. MANN. 1969. Vutersuchungen über die akute toxische Wirkung von Nitrilotriessigsäure (NTA). Arch. Fischereiwiss. 20: 178–181.

MURRAY, D., AND D. POVOLEDO. 1971. Determination of nitrilotriacetic acid in inland waters by gas chromatography of the trimethyl ester. J. Fish. Res. Board Can. 28: 1043–1047.

RENDER, F. W. 1970. Geohydrology of the metropolitan Winnipeg area as related to ground water supply and construction. Can. Geotec. J. 7: 243–274.

RUDD, J. W. M. 1972. The design and operation of a model aerated sewage lagoon. Fish. Res. Board Can. Tech. Rep. 294: 32 p.

RUDD, J. W. M., AND R. D. HAMILTON. 1972. Biodegradation of trisodium nitrilotriacetate in a model aerated sewage lagoon. J. Fish. Res. Board Can. 29: 1203–1208.

SPRAGUE, J. B. 1968. Promising anti-pollutant: chelating agent NTA protects fish from copper and zinc. Nature (London) 220: 1345–1346.

Similarity of Genic Polymorphism of Tetrazolium Oxidase in Bluefin Tuna (*Thunnus thynnus*) from the Atlantic Coast of France and the Western North Atlantic

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EDMUNDS, P. H., AND J. I. SAMMONS, III. 1973. Similarity of genic polymorphism of tetrazolium oxidase in bluefin tuna (*Thunnus thynnus*) from the Atlantic coast of France and the western North Atlantic. J. Fish. Res. Board Can. 30: 1031–1032.

Using electrophoretic techniques, we found three phenotypes of the enzyme tetrazolium oxidase in bluefin tuna (*Thunnus thynnus*) from the Bay of Biscay. The phenotypes were indistinguishable from those that in western Atlantic bluefin are controlled by two codominant, autosomal alleles. The frequency distributions of phenotypes in eastern and western Atlantic samples do not differ significantly from each other, nor does a frequency distribution of phenotypes in the combined sample differ significantly from a Hardy-Weinberg distribution. These facts support the hypothesis that bluefin tuna from the Bay of Biscay and from the western North Atlantic might belong to a single breeding population rather than to geographically discrete races. The facts are consistent with evidence from tagging, which shows sporadic transoceanic migration.

EDMUNDS, P. H., AND J. I. SAMMONS, III. 1973. Similarity of genic polymorphism of tetrazolium oxidase in bluefin tuna (*Thunnus thynnus*) from the Atlantic coast of France and the western North Atlantic. J. Fish. Res. Board Can. 30: 1031–1032.

Des méthodes électrophorétiques nous ont permis de déceler trois phénotypes de l'enzyme tétrazolium oxydase chez le thon rouge de la baie de Biscaye. Ces phénotypes ne diffèrent pas de ceux qui, chez le thon rouge de l'Atlantique occidental, sont contrôlés par deux allèles autosomiques codominants. La distribution des fréquences des phénotypes des échantillons des deux côtés de l'Atlantique ne diffère pas sensiblement l'une de l'autre. La distribution des fréquences des phénotypes d'un échantillon contenant un mélange des deux populations ne diffère pas non plus significativement d'une distribution de Hardy–Weinberg. Ces constatations confirment l'hypothèse selon laquelle les thons rouges de la baie de Biscaye et ceux de l'Atlantique Nord-Ouest appartiendraient à une même population reproductrice plutôt qu'à deux races géographiques discontinues. Ces faits sont également en accord avec les résultats du marquage, qui démontrent l'existence de migrations transocéaniques sporadiques.

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BLUEFIN tuna (*Thunnus thynnus*) from the western North Atlantic exhibit genic polymorphism of the enzyme tetrazolium oxidase. The polymorphism comprises three electrophoretic patterns: two (Ox-1A and Ox-1B) with a single band at either of two positions on the gel, and one (Ox-1AB) with three equidistant bands. Fish with either of the single-banded oxidase phenotypes presumably are homozygous for one of two codominant, autosomal alleles (Ox-1A and Ox-1B) controlling the variations, and fish with the triple pattern presumably are heterozygous (Edmunds and Sammons 1971).

In this note we report similar polymorphism among bluefin from the eastern North Atlantic and compare frequency distributions of Ox-1 phenotypes in the two areas.

Materials and methods — We obtained pieces of heart muscle from 675 bluefin tuna landed on June 30 and July 1, 1971, at St. Jean de Luz, France. Tissue samples were placed in plastic refrigerator bags and kept iced until their arrival at this laboratory on July 3, when they were frozen. Fork lengths of the fish, which were caught about 90 km WNW of St. Jean de Luz 1 or 2 days prior to landing, ranged from 75 to 92 cm, most being near 85 cm.

In our previous study of western Atlantic bluefin (Edmunds and Sammons 1971), tissues were homogenized in 0.2 M sucrose. In this study we used a less time-consuming technique that gave electrophoretically identical results: we diced small (0.1–0.2 g) chunks of

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TABLE 1. Observed distributions of Ox-1 phenotypes and (in parentheses) theoretical distributions based on inheritance controlled by codominant alleles.

Area	No. fish	Frequency of phenotype			P^a	χ^2	Probability of worse fit df = 1
		Ox-1A	Ox-1AB	Ox-1B			
Bay of Biscay	675	93 (89.4)	306 (312.5)	276 (273.0)	0.364	0.31	$0.75 > P > 0.50$
Western North Atlantic[b]	381	50 (48.0)	172 (174.5)	159 (157.7)	0.356	0.14	$0.75 > P > 0.50$
Total	1056	143 (138.4)	478 (487.8)	435 (429.8)	0.362	0.41	$0.75 > P > 0.50$

[a]Frequency of allele Ox-1A estimated from observed distributions of phenotypes.
[b]Data from Edmunds and Sammons (1971).

heart muscle and refrigerated them overnight in stoppered glass vials that contained 0.5 ml of a solution consisting of 85.6 g sucrose, 2.2 g K_2HPO_4, 14.5 g K_2HPO_4, 15 ml 2-phenoxyethanol, and water to make 1 liter. This formulation was used by Karig and Wilson (1971) in their electrophoretic studies of malate dehydrogenase in birds and reptiles, after Nakanishi et al. (1969) discovered that certain enzymes are liberated from tissues immersed in phenoxyethanol solutions.

Electrophoretic conditions were the same as in our previous study. We used 6% (w/v) acrylamide gels and a discontinuous buffer system: gel buffer contained 46 g of Tris (hydroxymethyl) amino methane (Tris)/liter and was adjusted to *p*H 8.9 with HCl; a *p*H 8.3 electrode buffer contained 0.6 g of Tris/liter and 2.9 g of glycine/liter. We electrophoresed 10-μliter aliquots of heart extracts at 350 v for about 3 hr, during which ethylene glycol at a temperature of 7 C was circulated through the electrophoretic cell to prevent overheating. Following electrophoresis, tetrazolium oxidase patterns were developed on the gel by incubating it in the dark for about 1 hr in 0.1 M Tris/HCl buffer (*p*H 8.0) that contained 6.5 mg of phenazine methosulfate/100 ml and 16 mg of the tetrazolium dye MTT/100 ml.

Results and discussion — The three tetrazolium oxidase phenotypes found in bluefin from the Bay of Biscay were indistinguishable from those found in bluefin from the western North Atlantic. Moreover, the observed frequency distribution of eastern Atlantic phenotypes does not differ significantly from a Hardy-Weinberg distribution (Table 1). It seems clear from these facts that Ox-1 variations of eastern Atlantic bluefin, like those of western Atlantic bluefin, are controlled by codominant, autosomal alleles.

The frequency distributions of Ox-1 phenotypes in samples from the two areas do not differ significantly (chi-square = 0.12, df = 2, $0.95 > P > 0.90$). Furthermore, the frequency distribution of phenotypes in the combined sample does not differ significantly from a Hardy-Weinberg distribution (Table 1). These observations support the hypothesis that bluefin tuna from the Bay of Biscay and the western North Atlantic belong to a single breeding population rather than to geographically discrete races, and are consistent with evidence from tagging (Mather 1969; F. J. Mather, III, personal communication), which shows sporadic transoceanic migration. However, the observations do not conclusively disprove that races exist.

EDMUNDS, P. H., AND J. I. SAMMONS. 1971. Genic polymorphism of tetrazolium oxidase in bluefin tuna, *Thunnus thynnus*, from the western North Atlantic. J. Fish. Res. Board Can. 28: 1053–1055.

KARIG, L. M., and A. C. WILSON. 1971. Genetic variation in supernatant malate dehydrogenase of birds and reptiles. Biochem. Genet. 5: 211–221.

MATHER, F. J., III. 1969. Long distance migrations of tunas and marlins. Underwater Natur. (Spring 1969): 6–14, 46.

NAKANISHI, M., A. C. WILSON, R. A. NOLAN, G. C. GORMAN, and G. S. BAILEY. 1969. Phenoxyethanol: protein preservative for taxonomists. Science 163: 681–683.

An Error in Logic in Estimating Stream Populations of Premigrant Trout from Stream Survival Rates and Lake Populations[1]

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IN a paper on the "Population ecology of brook trout, *Salvelinus fontinalis*, in Matamek Lake, Quebec," (Saunders and Power 1970) an attempt was made to provide a complete picture of the trout population, both those in the lake and in the nursery streams. It has since been discovered that the method developed to estimate the stream segment of the population is invalid.

Stream survival rates of young trout derived from rotenone samples and the estimated number of 3+ trout in the lake obtained from a mark and recapture experiment were used for the original calculation. The number of age 2+ trout required to yield the number of age 3+ trout in the lake was estimated using the formula $n = \frac{x}{y}$ where

n = the number of 2+ trout required
x = the number of age 3+ trout present in the lake
y = the annual survival rate of trout in the tributaries

Because the value of n represented the total number of 2+ trout required, the estimated number of 2+ trout in the lake was subtracted to obtain the number of age 2+ trout in the streams. From this estimate the numbers of trout in the various age groups in the tributaries were calculated using their annual survival rates. The 2+ trout in the stream, estimated by this method, are all required to give rise to the number of 3+ trout observed in the lake. The same trout cannot also give rise to the number of 3+ trout calculated to remain in the stream using the annual stream survival rate. It is here the error occurs.

The problem can be elaborated as follows. It was assumed that there were two types of trout inhabiting the streams around the lake, resident fish and those using the streams as nursery areas and originating from eggs deposited by fish which include lake residence in their life cycles. These types may interchange and are indistinguishable. Only the lake type was of interest, but the resident fish became involved when streams were sampled with rotenone. Ratios between age-groups in the streams, assuming populations of both types of fish remain stable from year to year, gave us an estimate of survival rate. The estimate was biased because of migration to the lake (and any sampling bias). Taking A as the number of premigrant 2+ trout and B the number of 3+ premigrant trout in the stream, while C is the number of 2+ trout and D the number of 3+ trout in the lake (in late summer when estimates were made), the following relationship applies: D = C multiplied by the survivorship between 2+ and 3+ in the lake plus the spring influx of 3+ migrants from the stream. The 3+ migrants are derived from 2+ premigrants in the stream. A segment of the matrix is shown below:

	Stream			
Age	*Resident*	*Migrant*		*Lake*
2+	X	A	→	C
	↓	↓		↓
3+	Y	B	→	D

So-called stream survivorship, $S = \frac{X + A}{Y + B}$ is influenced by migration. In the lake D>C, hence the only way an estimate of survivorship of C was possible was by making the doubtful assumption that lake survivorship L between 2+ and 3+ = S for the same interval.

$$A \text{ then } = \frac{D + B}{S} - C$$

[1]Correction to paper L. H. Saunders and G. Power entitled, "Population Ecology of Brook Trout, *Salvelinus fontinalis*, in Matamek Lake, Quebec." J. Fish. Res. Board Can. 27: 413–424.

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C, D, and S are known but with two unknowns the equation is insoluble except in the special case where B = zero. This is essentially what was assumed in the simplistic equation used in our paper, but it is invalid as trout are known to migrate to the lake between the ages of 1+ and 4+. To complicate the picture still further recent field work in the streams around Matamek Lake has shown maximum downstream movement of trout to the lake occurs at lowest water levels and may be a response to reduction in habitat at this time. Trout that end up in the lake may thus represent the surplus population of the streams during times of lowest carrying capacity.

The original publication may be reasonably correct, using as it does estimates of egg production as the starting point and valid estimates of the numbers of lake fish as an end point, but it seems advisable to point out the error and avoid further confusion.

SAUNDERS, L. H., AND G. POWER. 1970. Population ecology of the brook trout, *Salvelinus fontinalis*, in Matamek Lake, Quebec. J. Fish. Res. Board Can. 27: 413–424.

Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

(1) Fisheries Research Board of Canada
Biological Station
P.O. Drawer 100
Nanaimo, British Columbia

(2) Fisheries Research Board of Canada
Vancouver Laboratory
6640 N.W. Marine Drive
Vancouver 8, British Columbia

(3) Fisheries Research Board of Canada
Pacific Environment Institute
4160 Marine Drive
West Vancouver, British Columbia

(4) Fisheries Research Board of Canada
Freshwater Institute
501 University Crescent
Winnipeg, Manitoba R3T 2N6

(5) Fisheries Research Board of Canada
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Quebec

(6) Fisheries Research Board of Canada
Biological Station
St. Andrews, New Brunswick

(7) Fisheries Research Board of Canada
Marine Ecology Laboratory
Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia

(8) Fisheries Research Board of Canada
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(9) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

1789. KABATA, Z. 1972. *Alella* Leigh-Sharpe, 1925 (Crustacea: Copepoda): a request for designation of type-species Z. N. (S.) 2006. Bull. Zool. Nomencl. 29: 216–217. (1)

1790. MILLER, R. J., AND K. H. MANN. 1973. Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. III. Energy transformations by sea urchins. Mar. Biol. 18: 99–114. (7)

1791. CONOVER, R. J., AND C. M. LALLI. 1972. Feeding and growth in *Clione limacina* (Phipps), a pteropod mollusc. J. Exp. Mar. Biol. Ecol. 9: 279–302. (7)

1792. HUGHES, R. N., D. L. PEER, AND K. H. MANN. 1972. Use of multivariate analysis to identify functional components of the benthos in St. Margaret's Bay, Nova Scotia. Limnol. Oceanogr. 17: 111–121. (7)

1793. MANN, K. H., R. H. BRITTON, A. KOWALCZEWSKI, T. J. LACK, C. P. MATHEWS, AND I. MCDONALD. 1970. Productivity and energy flow at all trophic levels in the River Thames, England. Proc. of the IBP-UNESCO Symp. on Productivity Problems of Freshwaters, Kazimierz Dolny, Poland, May 6–12, 1970: 579–596. (7)

1794. TULL, C. E., P. GERMAIN, AND A. W. MAY. 1972. Mortality of thick-billed murres in the west Greenland salmon fishery. Nature 237: 42–44. (9)

1795. HARA, T. J. 1971. Sensory systems and electric organs, p. 79–121. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology, Vol. 5, Academic Press, New York, N.Y. (4)

1796. WOJTOWICZ, M. B., AND H. BROCKERHOFF. 1972. Isolation and some properties of the digestive amylase of the American lobster (*Homarus americanus*). Comp. Biochem. Physiol. 42B: 295–302. (8)

1797. ACKMAN, R. G., R. E. COX, G. EGLINTON, S. N. HOOPER, AND J. R. MAXWELL. 1972. Stereochemical studies of acyclic isoprenoid compounds I-Gas chromatographic analysis of stereoisomers of a series of standard acyclic isoprenoid acids. J. Chromatogr. Sci. 10: 392–401. (8)

1798. ACKMAN, R. G., L. SAFE, S. N. HOOPER, AND M. PARADIS. 1973. 7-methyl-7-hexadecenoic acid: isolation from lipids of the ocean sunfish *Mola mola* (Linnaeus) 1758. Lipids 8: 21–24. (8)

1799. PARSONS, T. R., C. D. MCALLISTER, R. J. LEBRASSEUR, AND W. E. BARRACLOUGH. 1972. The use of nutrients in the enrichment of sockeye salmon nursery lakes (A preliminary report), p. 1–7. *In* Marine pollution and sea

life. Fishing News (Books) Ltd., Surrey, England. (1)

1800. MCALLISTER, C. D., R. J. LEBRASSEUR, AND T. R. PARSONS. 1972. Stability of enriched aquatic ecosystems. Science 175: 562–564. (1)

1801. VERMEER, K., AND F. A. J. ARMSTRONG. 1972. Correlation between mercury in wings and breast muscles in ducks. J. Wildlife Manage. 36: 1270–1273. (4)

1802. MARGOLIS, L. 1970. The trematode *Plagioporus shawi* (McIntosh, 1939) n. comb. (Opecoelidae: Plagioporinae) from the north Pacific sockeye salmon, *Oncorhynchus nerka*. An. Inst. Biol. Univ. Nac. Auton. Mex. Ser. Zool. 41: 99–108. (1)

1803. ANTIA, N. J., R. S. KRIPPS, AND I. D. DESAI. 1972. L-threonine deaminase in marine planktonic algae. II. Disulfide and sulfhydryl group requirements of enzyme activity in two cryptophytes. J. Phycol. 8: 283–289. (2)

1804. WHYTE, J. N. C., AND G. A. STRASDINE. 1972. An intracellular α -D-glucan from *Clostridium botulinum*, type E. Carbohyd. Res. 25: 435–441. (2)

Additional FRB Publications

The Fisheries Research Board of Canada also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. *All publications may be referred to at Board establishments* located at: Ottawa; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

L'Office des recherches sur les pêcheries du Canada publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les librairies d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages *aux établissements de l'Office situés a* Ottawa (Ont.) à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

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Guide to Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

Editorial Practices

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970), Special Publication No. 6 of the American Fisheries Society.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

Series titles should be avoided. An *abstract* is required. Authors are encouraged to submit abstracts in both English and French; those submitted in one language only are translated by the *Journal*.

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and at least 1 mm high when the illustration is reduced to the appropriate size. Drawings should be grouped to facilitate comparisons. Originals must be submitted and should be less than 50 cm in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Photographs should be printed on glossy paper with a wide range in tone. They should be cropped, squared, and mounted on white cardboard, with no spaces between those arranged in groups.

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty *reprints*, without covers, are supplied free. Additional copies may be ordered when the galley proof is returned. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the biology?

Would any of the text be clearer if condensed? Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

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Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Les *notes* se distinguent des *articles* par la portée et le degré d'achèvement, plutôt que par la qualité des recherches. Les notes peuvent traiter de problèmes à portée restreinte et facilement définissables; de travaux qui en corroborent d'autres; d'engins et de méthodes de pêche, ou de techniques de recherche; de découvertes dérivées d'une recherche de plus grande envergure; de progrès accomplis dans l'exécution de projets abandonnés; enfin, de résultats préliminaires assez importants pour en justifier la publication avant la solution complète du problème. L'agencement des notes peut être aussi flexible que celui des articles, sauf qu'elles ne doivent pas avoir d'en-têtes de paragraphes centrés. La longueur ne doit pas dépasser quatre pages.

On doit en général éviter les *titres en séries*. L'auteur doit fournir un *résumé*. Si possible, le résumé devra être produit en anglais et en français; le *Journal* se charge de la traduction des résumés soumis en une seule langue.

Les *dessins au trait* doivent être exécutés de préférence à l'encre de Chine sur papier à dessin blanc, sur papier à calquer ou sur papier quadrillé bleu. Lignes, symboles, lettres et chiffres doivent être soignés et mesurer au moins 1 mm de hauteur lorsque la figure est réduite à la dimension voulue. On groupera les dessins de façon à faciliter les comparaisons. Les dessins originaux doivent accompagner le manuscrit, et ne doivent pas dépasser 50 cm dans le sens le plus long s'ils représentent une figure devant couvrir une page entière; ils seront proportionnellement plus petits pour des figures de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves de *photographies* doivent être tirées sur papier glacé à gamme de ton étendue. Elles doivent être coupées, dressées et montées sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

Les *épreuves en placards* sont adressées à l'auteur et il lui incombe de corriger toute erreur d'impression ou de contenu.

Cinquante *tirés à part*, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander des tirés à part supplémentaires. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscript, il doit spècifier que son institution accepte les frais.

Liste de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

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Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and another clear copy to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).
Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send the *original* and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also, type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral *one*, and between the capital letter O and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

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Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et une seconde copie suffisamment claire au bureau du Directeur de la publication.

Tableaux: Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).
Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par des minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Expédiez avec le manuscrit le *dessin original* et deux copies propres de chaque figure. Au coin inférieur gauche de chaque original et copie, inscrivez le nom de l'auteur et le numéro de la figure. De plus, annexez à chaque figure la légende qui lui est propre, pour fin d'identification. Dans le cas de photographies, dactylographiez l'information sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre l et le chiffre *un*, et entre la majuscule O et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le Journal (voir Appendice E). Les noms de revues sont abrégés suivant les *Biological Abstracts;* en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

Recent FRB Bulletins Available

No. 160. S. W. ROACH, H. L. A. TARR, N. TOMLINSON, AND J. S. M. HARRISON. 1967. Chilling and Freezing Salmon and Tuna in Refrigerated Sea Water, Fs 94–160, $1.75, 40 p.

No. 161. W. A. KENNEDY AND W. M. SPRULES. 1967. Goldeye in Canada, Fs 94–161, $1.50, 45 p.

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No. 164. N. M. CARTER. 1968. Index and List of Titles, Fisheries Research Board of Canada and associated Publications, 1900–1964, Fs 94–164, $1.00, 649 p.

No. 165. H. R. MCCRIMMON. 1968. Carp in Canada, Fs 94–165, $2.00, 93 p. (*also available in French*) *La Carpe au Canada.* 1972. Fs 94–165F, 101 p.

No. 166. J. G. EALES. 1968. The Eel Fisheries of Eastern Canada, Fs 94–166, $1.75, 79 p. (*also available in French*) *La pêche de l'anguille dans l'est du Canada.* 1972. Fs 94–166F, 89 p.

No. 167. J. D. H. STRICKLAND AND T. R. PARSONS. 1972. (Second edition), A Practical Handbook of Seawater Analysis, Fs 94–167, $7.50, 310 p. (first edition published in 1968).

No. 168. D. B. QUAYLE. 1969. Paralytic Shellfish Poisoning in British Columbia, Fs 94–168, $2.50, 68 p.

No. 169. D. B. QUAYLE. 1969. Pacific Oyster Culture in British Columbia, Fs 94–169, $5.00, 192 p.

No. 170. O. A. SÆTHER. 1969. Some Nearctic Podonominae, Diamesinae, and Orthocladadiinae (Diptera: Chironomidae), Fs 94–170, $3.00, 154 p.

No. 171. G. C. PIKE AND I. B. MACASKIE. 1969. Marine Mammals of British Columbia, Fs 94–171, $1.50, 54 p.

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No. 175. N. H. MORSE. 1971. An Economic Study of the Oyster Fishery of the Maritime Provinces, Fs 94–175, $2.25, 81 p.

No. 176. C.-T. SHIH, A. J. G. FIGUEIRA, AND E. H. GRAINGER. 1971. A synopsis of Canadian marine zooplankton, Fs 94–176, $5.00, 264 p.

No. 177. A. PRAKASH, J. C. MEDCOF, AND A. D. TENNANT. 1971. Paralytic shellfish poisoning in eastern Canada, Fs 94–177, $3.00, 87 p.

No. 178. D. B. QUAYLE. 1971. Pacific oyster raft culture in British Columbia, Fs 94–178, $2.00, 34 p.

No. 179. D. B. QUAYLE AND N. BOURNE. 1972. The clam fisheries of British Columbia, Fs 94–179, $3.00, 70 p.

No. 180. J. L. HART. 1973. Pacific Fishes of Canada, Fs 94–180, $8.00, 740 p.

Available by mail from Information Canada, Ottawa, and at the following Information Canada bookshops:

Halifax: *1687 Barrington Street*
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13th Pacific Science Congress

The Pacific Science Association, an international scientific organization, has announced the dates and tentative program of the 13th Pacific Science Congress. It will be held at the University of British Columbia from August 18 to 30, 1975. The Congress theme and related sub-themes are listed below; each of these will start with a Plenary Lecture to provide a comprehensive view of the topic, to be followed by several related symposia.

I. Pacific Population and Their Implications for Scientific Research
II. Nutrition
III. Energy Resources and Their Management
IV. Land-Based Resources and Their Management
V. Aquatic Resources and Their Management
VI. Man's Future Environment
VII. Science and Social Science Policy, Resources, and Strategies

Theme V on Aquatic Resources and Their Management embraces natural and man-induced changes in the aquatic resources of the Pacific Ocean area.

A. Plenary Lecture

Development of public policy for the rational use, distribution, and consumption of aquatic resources including coastline use, environmental protection, multi-use problems, and recreational development.

B. Symposia

1. Energy Transformation — Productivity of the Aquatic Environment.
Productivity of groups such as zooplankton and fish, as well as community analyses (benthos, neuston, etc.); productivity modelling.
2. Comparison of Fishery Research and Management Structure and Strategy of Pacific Rim Nations.
Effectiveness of various approaches including country or regional reviews.
3. State of Commercial Aquaculture.
Research and application on all aspects of freshwater, brackish, and marine aquatic husbandry.
4. Aquatic Pollution in the Pacific.
Discussion of pollutants.
5. Distribution of Biota in Relation to Oceanic Circulation.
Fundamental and applied aspects of oceanic, inshore, and estuarine circulation phenomena.
6. Education and Information Science in Aquatic Field.
Schools and training centers, data banks, and statistical papers.
7. Perspectives on the Pacific Aquatic Sciences, An Assessment of Lacunae in Fundamental and Applied Studies.
Reviews of Aquatic subjects (systematics, physiology, biogeography, geochemistry, oceanography, limnology, etc.).

C. Contributed Papers

Related to the symposia themes and providing more detailed and technical information.

Further information and subsequent announcements may be obtained from Dr. W. S. Hoar, Secretary-General, University of British Columbia, Vancouver 8, Canada.

Fisheries Research Board of Canada Publications

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Miscellaneous Special Publication

Index and List of Titles, Fisheries Research Board of Canada and Associated Publications, 1965–1972.

Neal M. Carter

Journal of the Fisheries Research Board of Canada (Special issue) (Vol. 30, No. 12, Part 2, 1973)

Proceedings of the Technical Conference on Fishery Management and Development, Vancouver, Canada, February 13–23, 1973 (Food and Agricultural Organization of the United Nations)

Special editors: W. E. Ricker and E. P. Weeks

Journal of the Fisheries Research Board of Canada

Volume 30, No. 7, July 1973 JFRBA30(7)867-1037(1973)

Aspects saisonniers de la fixation de l'épifaune benthique de l'étage infralittoral de l'estuaire du Saint-Laurent — E. Bourget et G. Lacroix — 867–880

Life cycle of *Salmincola californiensis* (Dana 1852) (Copepoda: Lernaeopodidae) — Z. Kabata and B. Cousens — 881–903

Effects of temperature on responses of the gonads of green sunfish (*Lepomis cyanellus*) to treatment with carp pituitaries and testosterone propionate — C. M. Kaya — 905–912

An established cell line from the Atlantic salmon (*Salmo salar*) — B. L. Nicholson and C. Byrne — 913–916

Some comparisons in the thermal structure of lakes Wood, Kalamalka, Okanagan, Skaha, and Osoyoos, British Columbia — J. O. Blanton — 917–925

Identification of supernatant and mitochondrial isozymes of malate dehydrogenase on electropherograms applied to the taxonomic discrimination of walleye (*Stizostedion vitreum vitreum*), sauger (*S. canadense*), and suspected interspecific hybrid fishes — J. W. Clayton, R. E. K. Harris, and D. N. Tretiak — 927–938

Delay-differential equation models for fisheries — G. G. Walter — 939–945

Observations on the cestode *Eubothrium salvelini* in juvenile sockeye salmon (*Oncorhynchus nerka*) at Babine Lake, British Columbia — H. D. Smith — 947–964

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Chemical basis for homing of Atlantic salmon (*Salmo salar*) to a hatchery — A. M. Sutterlin and R. Gray — 985–989

Notes

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